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*Aquatic Plant Control Research Program*

# **Utilization of Herbicide Concentration/Exposure Time Relationships for Controlling Submersed Invasive Plants on Lake Gaston, Virginia/North Carolina**

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**Abstract:** Lake Gaston is a large, multiple purpose impoundment on the Roanoke River between eastern North Carolina and Virginia. Invasive plants have been increasing on the lake since 1982. By 2002, over 1,200 ha of the lake were infested with several invasive plants, and an integrated management program (herbicides and grass carp) was underway. To improve herbicide performance on the lake, this study focused on three phases for controlling the lake's invasive submersed vegetation (the plants targeted were monoecious and dioecious hydrilla; Eurasian watermilfoil; egeria; and the bluegreen alga, lyngbya; non-target plants evaluated were vallisneria and southern naiad.) Phase one summarizes herbicide dose-response interactions (concentration and exposure time (CET) relationships) for controlling these plants using older aquatic herbicides; phase two evaluates CET relationships for new aquatic herbicides; and phase three provides interim management guidance for Lake Gaston.

Product-specific CET information is best utilized when combined with site-specific water exchange patterns found in plant stands targeted for chemical applications. Prescriptive treatments can then be developed to selectively remove invasive plants. Results from evaluations showed that control of target plants was dependent upon product specific herbicide CET relationships, with efficacy ranging from poor to excellent.

Information provided in this report can be used for developing prescriptive treatment strategies for selectively controlling invasive plants on Lake Gaston. Recommendations for specific herbicides should be viewed as a "best fit" based on current information. This interim chemical control guidance should be refined once site-specific water exchange processes are determined for treatment sites on Lake Gaston.

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## Preface

The work reported herein was conducted as part of the Aquatic Plant Control Research Program (APCRP). The APCRP is sponsored by Headquarters, U.S. Army Corps of Engineers (HQUSACE), and is assigned to the U.S. Army Engineer Research and Development Center (ERDC) under the purview of the Environmental Laboratory (EL), Vicksburg, MS. Funding was provided under Department of the Army Appropriation No. 96X3122, Construction General. Support was also provided by the U.S. Army Engineer District, Wilmington, the Lake Gaston Weed Control Council, and the Aquatic Ecosystem Restoration Foundation. The APCRP is managed under the Civil Works Environmental Engineering and Sciences office, Dr. Alfred F. Cofrancesco, Technical Director. Dr. Linda S. Nelson was Program Manager for the APCRP. Technical Monitor during this study was Timothy R. Toplisek, HQUSACE.

The Principal Investigator of this work was Dr. Kurt D. Getsinger, Environmental Processes Branch (EPB), Environmental Processes and Engineering Division (EPED), EL. This work was conducted and the report prepared by Dr. Getsinger, Angela G. Poovey, and LeeAnn Glomski, EPB; Jeremy G. Slade, Center for Aquatic and Invasive Plants, University of Florida, Gainesville, FL; and Dr. Robert J. Richardson, Department of Crop Science, North Carolina State University, Raleigh, NC.

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Technical reviews of this report were provided by Dr. Linda S. Nelson and Dr. Judy Shearer, ERDC. This work was performed under the general supervision of Dr. Beth Fleming, Director, EL; Warren Lorentz, Chief, Environmental Processes and Engineering Division (EPED); and Mark Farr, Chief, Environmental Processes Branch (EPB). At the time of



publication of this report, Dr. Jeffery P. Holland was Director of ERDC. COL Kevin J. Wilson was Commander and Assistant Director.

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# 1 Introduction

## Background

Lake Gaston is a large impoundment of over 8,100 hectares (ha) (or 20,000 acres) on the Roanoke River. It is located within the boundaries of Warren, Halifax, and Northampton counties in North Carolina, and Brunswick and Mecklenburg counties in Virginia. Lake Gaston is well utilized; it is used to generate electricity by Dominion Power, for recreation by the public (e.g. for boating, fishing and hunting), and it serves in a flood control capacity for the Roanoke River Valley. The reservoir also provides a source of high-quality potable water for cities in the region. Aquatic weed problems on Lake Gaston have been increasing ever since the first report of the invasive macrophyte, egeria, in 1982. By 2002, over 1,200 ha of the lake had become infested with a variety of invasive plants, including egeria, hydrilla, Eurasian watermilfoil, and lyngbya, and an integrated management program using herbicides and grass carp was underway.

In an effort to improve the species-selective use of herbicides on the lake, two levels of chemical control work were proposed for Lake Gaston: small-scale baseline studies and field-level demonstrations. Of the chemical control work proposed for Lake Gaston, this document reports on findings from the small-scale baseline assessments and studies. These efforts focused on assessing herbicide dose-response interactions, also known as concentration and exposure time (CET) relationships. Linking results of the small-scale evaluations with results from field-level demonstrations can provide information required to develop prescriptive herbicide treatments for controlling the major submersed invasive plants on the lake, while minimizing damage to beneficial native vegetation.

Using small- and mesocosm-scale experimental systems, herbicide CET studies have demonstrated that effective long-term weed control is dependent upon the length of time plants remain exposed to given concentrations of herbicide against various aquatic species (Figure 1). Results from these studies provide evidence of efficacy in controlling invasive plant species, and are verified in aquatic and wetland field sites throughout the US. This multi-tiered approach has proven invaluable in the successful use of aquatic herbicides in the field (Poovey and Getsinger 2005). Once the concept of linking CET relationships with improved

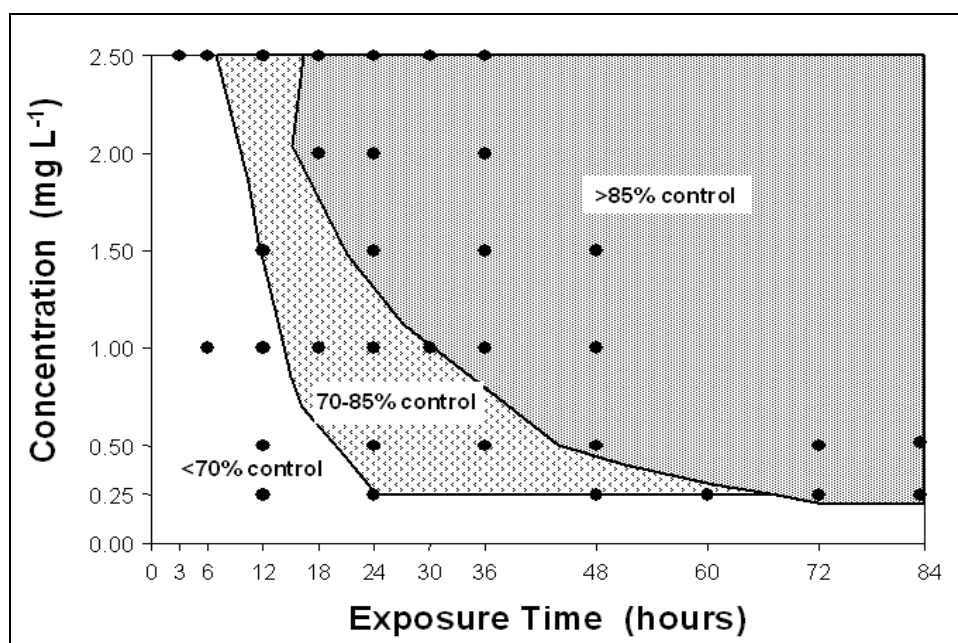


Figure 1. Generalized herbicide concentration and exposure time relationships for controlling submersed plants.

control became empirically established (Getsinger and Netherland 1997), it allowed for the development of species-selective application techniques for submersed weeds, and set the stage for prescriptive treatment strategies.

Through rigorous experimentation, it became clear that chemicals generally regarded as “broad spectrum” products, that is, they controlled all types of vegetation, could be species-selective based on the rate of application (Getsinger et al. 2001, Parsons et al. 2001, Getsinger et al. 2002, Skogerboe and Getsinger 2002). Other studies revealed that by understanding the phenological events of the targeted invasive plants, along with the life-cycle patterns of the non-target native vegetation, innovative management strategies could provide species-selective control based on timing of application and CET information (Poovey et al. 2002, Pedlow et al. 2006). Consequently, determining CET relationships has become an accepted and important phase in the pursuit of screening and evaluating new aquatic herbicides. However, sustained lines of CET research may take many years, and are directly related to a number of factors, such as herbicide mode-of-action, environmental fate and dissipation of the herbicide molecule, life cycle of target and non-target plants, and levels of resources applied to the efforts.

## Objectives

This study focuses on three phases relating to the selective control of the invasive submersed vegetation that currently infests Lake Gaston: monoecious and dioecious hydrilla (*Hydrilla verticillata* (L.f.) Royle); Eurasian watermilfoil (*Myriophyllum spicatum* L); egeria (*Egeria densa* Planch); and the filamentous bluegreen alga, lyngbya (*Lyngbya* spp. Agardh). The first phase reviews and summarizes CET relationships for controlling these plants using currently registered aquatic herbicides. The second phase evaluates CET relationships for new aquatic herbicides under registration review. The third phase provides interim guidance about the use of these aquatic herbicides on Lake Gaston.

## 2 Review and Summary of Herbicides

### CET Relationships

Critical CET relationships were summarized and/or evaluated for selected herbicides against four invasive species growing in Lake Gaston: the submersed macrophytes monoecious hydrilla, Eurasian watermilfoil, and egeria, and the bluegreen alga, lyngbya. The desirable non-target, native plants vallisneria, (*Vallisneria americana* Michx) and southern naiad (*Najas guadalupensis* (Sprengel) magnus) were also evaluated as part of this work.

Herbicides included old and new chemistries (both contact-type and systemic) and were grouped based upon US Environmental Protection Agency (USEPA) registration status in 2006: (a) currently registered for aquatic sites with Section 3 (US-wide) labels, and (b) new products that are being evaluated for aquatic use under Section 18-Emergency Exemption (EE), Section 24C-Special Local Needs (SLN), and/or Section 5-Experimental Use Permit (EUP) labels. An overview of selected aquatic herbicide classifications, modes of action and general use patterns are provided in Tables 1 and 2.

For some of the currently registered aquatic herbicides assessed in this study, CET relationships were summarized from previously published work conducted, or contracted by, the U.S. Army Engineer Research and Development Center (ERDC). These CET relationships are being utilized in the field; however, some refinement may be required as new information becomes available. Where CET information was lacking for currently registered chemistry, or for new products under registration review, a series of small-scale studies were conducted to fill in data gaps. The small-scale studies were conducted in a variety of laboratory, growth chamber and green house facilities located at the ERDC, Vicksburg, MS; the ERDC Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, TX; and the Department of Crop Science, North Carolina State University (NCSU), Raleigh, NC.

Contact herbicides are products that have a broad spectrum of activity and can be used to control most submersed plant species. Knowledge of CET relationships with respect to contact herbicides can be used to provide

some degree of species selectivity. In addition, the active ingredients in these products do not translocate throughout the plant, and therefore only affect the tissue that is contacted by the herbicide. With the exception of annual plants and very young perennial plants (with poorly developed rootstock or rootcrown tissue), contact herbicides rarely kill the entire plant. When used to control submersed vegetation, they perform well in removing or “burning-down” the shoots, but do not control the rootstock or rootcrown tissue which is at or below the surface of the sediment. Because of this, robust perennial species such as Eurasian watermilfoil

**Table 1. Section 3 herbicides for control of the submersed plants monoecious hydrilla, Eurasian watermilfoil, and egeria.**

Compound	Formulation	Mode of Action	Application Rate	Exposure Time	Application Sites	Plant Response
<b>Contact</b>						
Carfentrazone-ethyl	Liquid	Blocks chlorophyll synthesis	100-200 $\mu\text{g L}^{-1}$	12-36 hours	Shoreline, localized treatment sites, moving or still water.	Chlorosis of stems and leaves with plant death in 3-7 days.
Diquat	Liquid	Destroys cell membranes	0.185-0.37 $\text{mg L}^{-1}$	6-36 hours	Shoreline, localized treatment sites, moving or still water.	Chlorosis of stems and leaves with plant death in 3-7 days.
Endothall <sup>1</sup>	Liquid or granular	Inhibits respiration	1-5 $\text{mg L}^{-1}$	12-72 hours	Shoreline, localized treatment sites, moving or still water.	Defoliation and browning of stems with plant death in 2 to 4 weeks.
<b>Systemic</b>						
Chelated Copper <sup>2</sup>	Liquid	Interferes with photosynthesis	0.25-1 $\text{mg L}^{-1}$	3-24 hours	Shoreline, localized treatment sites, moving or still water.	Chlorosis of stems and leaves with plant death in 7-10 days.
2,4-D <sup>3</sup>	DMA liquid, BEE salt granular	Causes uncontrolled plant-growth	0.5-4 $\text{mg L}^{-1}$	12-72 hours	Bays, coves and areas with slow-moving or still water.	Stem twisting and leaf curling with plant death in 3-5 weeks.
Fluridone	Liquid or granular	Prevents carotene synthesis	5-15 $\mu\text{g L}^{-1}$	60-90 days	Bays, coves and areas with slow-moving or still water.	Chlorosis of stems with plant death in 8-12 weeks.
Triclopyr <sup>3</sup>	Liquid or granular	Causes uncontrolled plant growth	0.5-2.5 $\mu\text{g L}^{-1}$	12-72 hours	Bays, coves and areas with slow-moving or still water.	Stem twisting and leaf curling with plant death in 3-5 weeks.

<sup>1</sup> Not recommended for egeria control.

<sup>2</sup> Nor recommended for Eurasian watermilfoil control.

<sup>3</sup> Not recommended for hydrilla or egeria control.

**Table 2. Herbicides under special registrations for control of algae and the submersed plants monoecious hydrilla, Eurasian watermilfoil, and egeria.**

Compound	Formulation	Mode of Action	Estimated Application Rate	Exposure Time	Potential Application Sites	Expected Plant Response
<b>Contact</b>						
Flumioxazin	Water Dispersible Granule	Blocks chlorophyll synthesis	100-400 $\mu\text{g L}^{-1}$	12-36 hours	Shoreline, localized treatment sites, moving or still water.	Chlorosis of stems and leaves with plant death in 3-7 days.
<b>Systemic</b>						
Bispyribac-sodium	Water Dispersible Granule	Disrupts protein synthesis	10-200 $\mu\text{g L}^{-1}$	45-90 days	Bays, coves and areas with slow-moving or still water.	Chlorosis and reddening of stems and leaves with plant death in 6-12 weeks.
Imazamox	Liquid	Disrupts protein synthesis	10-200 $\mu\text{g L}^{-1}$	45-90 days	Bays, coves and areas with slow-moving or still water.	Chlorosis and reddening of stems and leaves with plant death in 6-12 weeks.
Penoxsulam	Liquid	Disrupts protein synthesis	10-200 $\mu\text{g L}^{-1}$	45-90 days	Bays, coves and areas with slow-moving or still water.	Chlorosis and reddening of stems and leaves with plant death in 6-12 weeks.

and hydrilla that are treated with contact herbicides usually have the ability to recover from the herbicide exposure and regrow. Contact herbicides typically provide maximum control during the year of treatment. Contact herbicides assessed in this report are carfentrazone-ethyl, chelated copper, diquat, endothall, and flumioxazin.

Systemic herbicides, unlike contact herbicides, translocate throughout the plant and under ideal conditions can provide complete control of the target weed. These herbicides are primarily absorbed by the leaf and stem tissues and move to the actively growing apical regions of roots and shoots, with the potential of killing the entire plant. Generally, systemic herbicides are most effective when applied early in the growth cycle of target weeds, so that lower rates can be used. Systemic herbicides typically provide maximum control beyond the year of treatment. Systemic herbicides assessed in this report are 2,4-D, triclopyr, fluridone, imazamox, bispyribac-sodium, and penoxsulam.

## Plants and Herbicides Evaluated

For organizational purposes, results of herbicide CET assessments (from previous and current studies) provided in this report are grouped by target plant species and herbicide, and include:

1. Monoecious and dioecious hydrilla – diquat, endothall, fluridone, carfentrazone-ethyl, flumioxazin, imazamox, and penoxsulam;
2. Eurasian watermilfoil – carfentrazone-ethyl, diquat, endothall, fluridone, triclopyr, 2,4-D, flumioxazin, bispyribac-sodium, imazamox, and penoxsulam;
3. Egeria – diquat;
4. Other submersed macrophytes: vallisneria, southern naiad - endothall, triclopyr, fluridone, bispyribac-sodium, imazamox, and penoxsulam;
5. Lyngbya – chelated coppers and endothall.

Section 3 aquatic herbicides assessed included:

1. carfentrazone-ethyl (a,2-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoic acid, ethyl ester
2. chelated copper (ethanolamine or ethylenediamine copper complexes)
3. diquat (6,7-dihydrodipyrido[1,2-a:2',1'-c]pyrazinediium ion
4. endothall (7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid)
5. fluridone (1-methyl-3phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone)
6. triclopyr [(3,5,6-trichloro-2-pyridinyl)oxy]acetic acid
7. 2,4-D (2,4-dichlorophenoxy acetic acid)

Assessment of new products included:

1. bispyribac-sodium (2,6-bis[(4,6-dimethoxy-2-pyrimidinyl)oxy]benzoic acid); Section 5-EUP.
2. flumioxazin (2-[7-fluoro-3,4-dihydro-3-oxo-4-(2-proynyl)-2H-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1H-isoindole-1,3(2H)-dione); Section 5-EUP
3. imazamox (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-(methoxymethyl)-3-puridinecarboxylic acid, ammonium salt; Section 24C-SLN, Section 5-EUP.
4. penoxsulam (2-(2,2-difluoroethoxy)-6-trifluoromethyl-N-(5,8-dimethoxy[1,2,4]triazolo-[1,5c]pyrimidin-2-yl)benzenesulfonamide); Section 18-EE, Section 5-EUP.



### **3 Plant Species Evaluations**

#### **Monoecious Hydrilla – Nuisance Exotic Species**

Summarized results from previous studies using diquat, endothall, and fluridone are provided below:

##### **Diquat**

Results from laboratory CET evaluations demonstrated that diquat (as Diquat®, Ortho Chemical Co., CA, currently formulated as Reward®, Syngenta Professional Products, Greensboro, NC) applied at rates of 0.25 to 2.0 mg/L for contact times of 6 to 48 h provided up to 80% control of monoecious hydrilla (Van and Connant 1988). Young plants sprouted from tubers were more susceptible to the diquat CETs than plants grown from apical shoot cuttings.

##### **Endothall**

Results from laboratory CET evaluations showed that endothall (as Aquathol®, United Phosphorus International, King of Prussia, PA) applied at rates of 1 to 5 mg/L for contact times of 3 to 168 h provided up to 83 % control of monoecious hydrilla (Van and Connant 1988). Susceptibility of young plants sprouted from tubers to the diquat CETs tested was similar to responses measured in plants grown from apical shoot cuttings.

##### **Fluridone**

Results from laboratory CET evaluations determined that fluridone (as Sonar® 4AS, SePRO Corporation, Carmel, IN) applied at rates of 0.1 to 1 mg/L for contact times of up to 10 d provided 90 % control of monoecious hydrilla after only four days of exposure (Van and Connant 1988). Susceptibility of young plants (sprouted from tubers) to the fluridone CETs was similar to that in plants grown from apical shoot cuttings.

#### **Current Evaluations to Determine CET Information**

Herbicide CETs evaluated in this section (Studies 1 through 4) are for the contact-type compounds, endothall, carfentrazone-ethyl and flumioxazin, and for the systemic compound, penoxsulam.

## **Study 1 (A and B). Monoecious and Dioecious Hydrilla Treated with Endothall**

**Objectives:** To evaluate the efficacy of endothall against monoecious hydrilla grown from different plant structures (shoots vs. rootcrowns), under various concentrations and exposure times; to compare the results with similar studies conducted with dioecious hydrilla.

### **Study 1A – Plants Grown From Shoot Cuttings.**

#### **Materials and Methods**

This study was conducted in a controlled-environment growth chamber (58 m<sup>2</sup>) at the ERDC with an air temperature of 21 ± 2°C, light intensity of 520 ± 50 µmol m<sup>-2</sup> sec<sup>-1</sup>, and photoperiod of 14 h:10 h light: dark cycle. Lighting was provided by a combination of 400 watt high-pressure sodium and metal halide bulbs. Experimental conditions within the chamber were maintained to simulate ambient conditions conducive for submersed plant growth.

Four healthy apical cuttings (15 cm) of monoecious hydrilla (collected from North Carolina) were planted in 300 ml plastic beakers (diameter=7 cm, depth=12 cm) filled with lake sediment and amended with 150 mg L<sup>-1</sup> ammonium chloride. A 1-cm layer of silica sand was added to the sediment surface to prevent suspension of sediment particles in the water column. Four beakers were placed in each of the 28 (48-L) aquaria. All aquaria were then filled with growth solution (Smart and Barko 1985) specific for growth and establishment of aquatic plants. Dioecious hydrilla was collected from Florida and planted as described above. Four beakers of the dioecious biotype were placed side-by-side with the monoecious biotype in each aquarium; consequently, each aquarium had a total of eight planted beakers, four of each biotype.

Monoecious plants grew for 28 d while dioecious plants grew for 21 d, at which time both biotypes were actively growing and forming a surface canopy. One day before herbicide application, four planted beakers of each biotype were randomly sampled for a pretreatment biomass. Shoots were clipped at the sediment surface, dried at 70°C for 48 h to obtain a dry weight (DW). Pretreatment biomass (mean ± 1 SE, *n*=4) for monoecious hydrilla was 0.48 ± 0.09 g and for 1.16 ± 0.13 g dioecious hydrilla. The monoecious biotype had shoot biomass within the range of spring biomass

in North Carolina (Harlan et al. 1985), while the dioecious biotype had biomass shoot biomass within the range of spring biomass in Florida (Bowes et al. 1979).

For herbicide application, a stock solution of endothall (formulated as Aquathol® K) was prepared as 5.07 g active ingredient (ai) L<sup>-1</sup> based on the dipotassium salt. Herbicide rates of 1, 2, and 4 mg ai L<sup>-1</sup> were used with exposure times ranging from 24 to 96 h to determine CET relationships. Untreated reference aquaria were included to assess plant growth in the absence of herbicide exposure. After each exposure time, aquaria were drained and filled twice with growth solution.

Water samples were collected from one treatment replicate at 24 h after treatment to ensure nominal herbicide concentrations were achieved. Samples were stored at 4°C until shipped for analysis. Samples were analyzed using the enzyme-linked immunosorbent assay (ELISA) technique. Water temperature was measured continuously with an Optic Stowaway® Temperature Probe (Onset Computer Corp., Bourne, MA) in aquaria. Aqueous pH was measured at the beginning and end of the study with a WTW pH 315i meter (WTW Measurement Systems, Ft. Meyers, FL).

Herbicide efficacy was assessed at 6 wk after treatment by harvesting shoot biomass. Shoots were clipped at the sediment surface, dried at 70°C for 48 h to obtain a DW. Each treatment-- including the untreated reference -- was replicated three times. Data were compared to the reference and calculated as percent control. Data were then subjected to a one-way analysis of variance (ANOVA) based on ranks to determine herbicide effects. If effects were significant ( $p \leq 0.05$ ), means were compared using the Student-Newman-Keuls method (S-N-K).

## Results and Discussion

During the study, water temperatures (mean  $\pm 1$  SE) in the aquaria ranged from 20.4 to 23.2 °C, while the pH ranged from 8.6 to 9.1. Water residues (mean  $\pm 1$  SE) were  $1.03 \pm 0.03$  mg ai L<sup>-1</sup> ( $n=6$ ),  $2.23 \pm 0.03$  mg ai L<sup>-1</sup> ( $n=9$ ), and  $4.28 \pm 0.13$  mg ai L<sup>-1</sup> ( $n=9$ ) for the nominal concentrations of 1, 2, and 4 mg ai L<sup>-1</sup>, respectively.

### *Dioecious hydrilla*

Control of dioecious hydrilla was similar for all treatments (Figure 2A), even though percent control of 1 mg ai L<sup>-1</sup> with a 48-h exposure time

(1/48) was only  $36.8 \pm 12.9\%$ . Control at  $1 \text{ mg ai L}^{-1}$  with a 96-h exposure time (1/96) was measured at  $86.3 \pm 11.8\%$ . Some shoots were still green and healthy with new growth present for the 1/48 treatment, compared to brown and decaying shoots for the 1/96 treatment. Although the 1/48 treatment provided partial control ( $<50\%$ ), plants from this treatment would probably recover based on the CET relationships documented by Netherland et al. (1991), who used the same study system. Percent control for the other endothall treatments ranged from 90 to 100%, with no visual evidence of shoot recovery potential. Control of these treatments matched that predicted by the endothall-hydrilla CET relationships developed by Netherland et al. (1991) and results from other studies (Pennington et al. 2001, Skogerboe and Getsinger 2001).

#### *Monoecious hydrilla*

Control of monoecious plants dosed with 1 and  $2 \text{ mg ai L}^{-1}$  for a 48-h exposure time was  $32.3 \pm 10.8\%$  and  $59.8 \pm 9.31\%$ , respectively (Figure 2B). Control from these treatments was significantly lower than the other endothall treatments, which ranged from 84 to 100%. In the 1/48 treatment, most of the hydrilla shoots were still green and healthy with new growth present, compared to brown and decaying shoots for the 2/48 treatment. Like the dioecious hydrilla, the monoecious hydrilla from the 1/48 treatment would probably recover, while plants from the 2/48 treatment probably would not survive. The 4/24 and 2/72 treatments provided  $84.2 \pm 2.45$  and  $88.1 \pm 6.48$  percent control, respectively. Although these treatments were significantly lower than the 4/72 treatment, which had 100% control, the 4/24 and 2/72 treatments were successful because there would be few viable stems present as a source of potential regrowth. Treatments that provided 90 to 100% control were the 1/96, 2/96, 4/48, 4/72. Control of these treatments matched that predicted by the endothall CET relationships generated for dioecious hydrilla by Netherland et al. (1991).

Results from this study showed that endothall is efficacious against monoecious hydrilla. It provides excellent control with low concentrations ( $2 \text{ mg ai L}^{-1}$ ) when coupled with adequate exposure times (72 to 96 h). These findings are consistent with efficacy rates reported for endothall against the dioecious biotype of hydrilla, reported in this study and others. Investigation of higher concentrations with shorter exposure times is needed to determine whether endothall would be effective in areas with high water exchange on Lake Gaston.

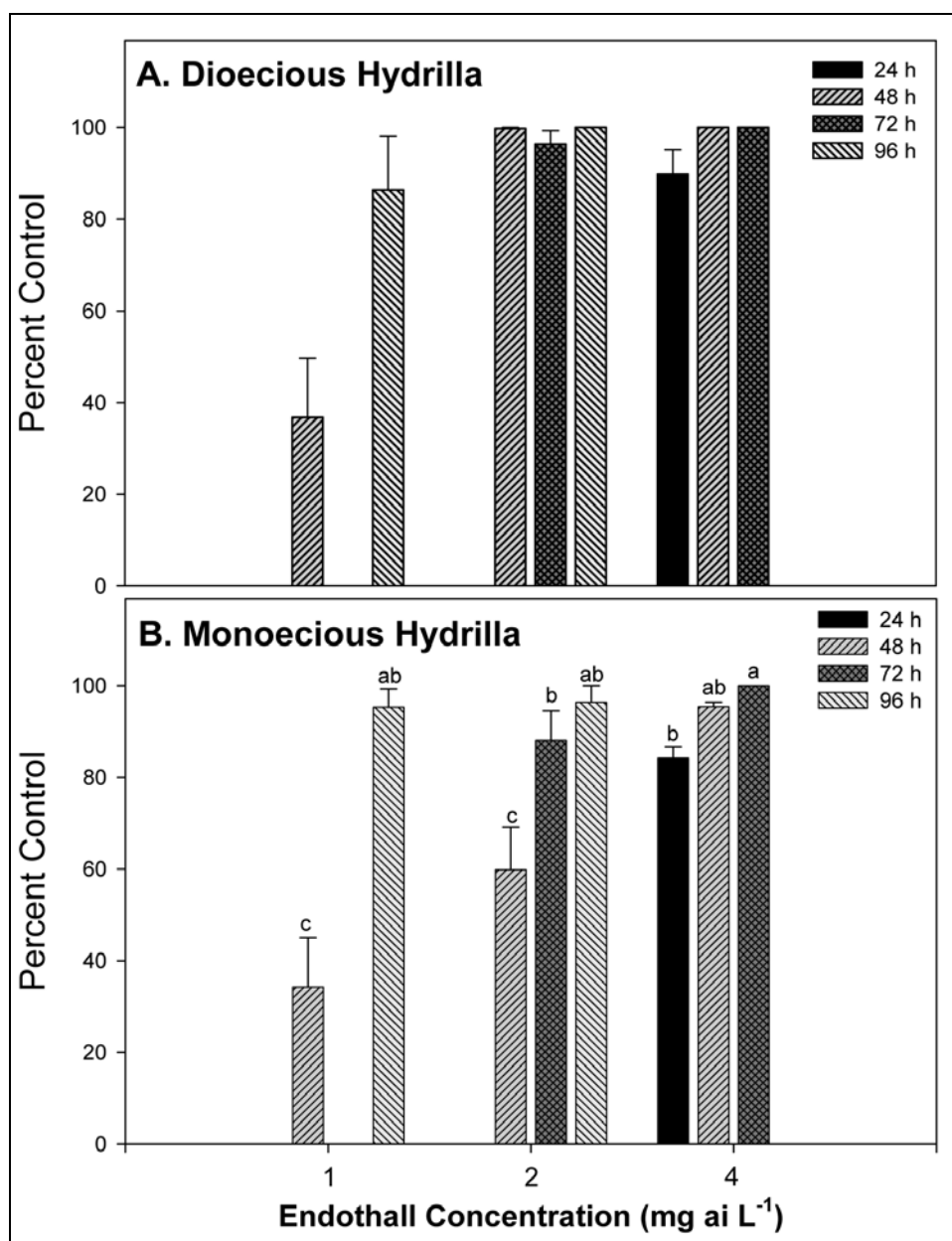


Figure 2. Percent control of A) dioecious and B) monoecious hydrilla grown from shoot clippings using 1, 2, and 4 mg ai L<sup>-1</sup> endothall under various exposure periods (24, 48, 72, and 96 h). Means are  $\pm 1$  SE ( $n=3$ ). Treatments with different letters are significantly different (S-N-K,  $p \leq 0.05$ ).

## Study 1B – Plants Grown From Tubers.

### Materials and Methods

This study was conducted in a controlled environment growth chamber at the ERDC under conditions described for Study 1A, above. Tubers of monoecious hydrilla were collected from North Carolina and refrigerated at 4 °C for 2 d. Afterwards, tubers were placed in reverse osmotic (RO) water,

aerated, and allowed to sprout for 3 wk. Mean tuber weight was  $0.14 \pm 0.01$  g fresh weight (FW;  $n=24$ ). Three sprouted tubers (shoot length =  $3.32 \pm 0.12$  cm) were planted in 300 ml glass beakers (diameter = 7 cm, depth = 12 cm) filled with natural lake sediment amended with  $150 \text{ mg L}^{-1}$  ammonium chloride. A 1-cm layer of silica sand was added to the sediment surface to prevent suspension of sediment particles in the water column. Three beakers were placed in each of the 24 (10-L) aquaria. All aquaria were then filled with growth solution (Smart and Barko 1985) specific for growth and for the establishment of aquatic plants. Tubers of dioecious hydrilla were collected from Florida. Mean tuber weight was  $0.47 \pm 0.05$  g FW ( $n=24$ ). Tubers were sprouted for 3 d, then planted as described above (shoot length =  $15.0 \pm 2.20$  cm). Three beakers of the dioecious biotype were placed in each of 21 (10-L) aquaria. Therefore, there were 45 total aquaria: 24 aquaria planted with monoecious hydrilla and 21 aquaria planted with dioecious hydrilla. Each aquarium contained three planted beakers.

Monoecious plants grew for 7 wk while dioecious plants grew for 5 wk. Plants were actively growing and forming a surface canopy prior to herbicide application. One day before herbicide application, one beaker was removed from each aquarium for a biomass estimate. Shoots were clipped at the sediment surface and dried at  $70^\circ\text{C}$  for 48 h to obtain g DW. Pre-treatment DW (mean  $\pm 1$  SE) for monoecious hydrilla was  $0.43 \pm 0.07$  g ( $n=24$ ) and  $0.32 \pm 0.03$  g ( $n=21$ ) for dioecious hydrilla. The monoecious biotype had shoot biomass within the range of spring biomass in North Carolina (Harlan et al. 1985), while the dioecious biotype had shoot biomass within the range of early spring biomass in Florida (Bowes et al. 1979).

For herbicide application, a stock solution of endothall (formulated as Aquathol® K) was prepared as  $5.07 \text{ g ai L}^{-1}$  based on the dipotassium salt. Herbicide rates of 1, 2, and  $4 \text{ mg ai L}^{-1}$  were used with exposure times ranging from 24 to 96 h to determine CET relationships. Untreated reference aquaria were included to assess plant growth in the absence of herbicide exposure. After each exposure time, the aquaria were drained and filled twice with growth solution to remove any herbicide residues.

Water samples were collected from random aquaria at 24 h after treatment to ensure nominal herbicide concentrations were achieved. Samples were stored at  $4^\circ\text{C}$  until shipped for analysis. Samples were analyzed using the ELISA technique. Water temperature was measured continuously in the

aquaria with the probe described above. The pH was measured at the beginning and end of the study with the same probe.

Herbicide efficacy was assessed at 6 wk after treatment by harvesting shoots. Shoots were clipped at the sediment surface and dried at 70°C for 48 h to obtain g DW. Each treatment, including the untreated reference, was replicated three times. A one-way ANOVA was conducted to determine herbicide effects on percent control based on shoot dry weights. If the main effects were significant ( $p \leq 0.05$ ), means were compared using the S-N-K method.

## Results and Discussion

During the study, water temperatures (mean  $\pm 1$  SE) in the aquaria were  $22.38 \pm 0.04$  °C, while pH was  $9.0 \pm 0.1$ . Water residues (mean  $\pm 1$  SE) were  $0.97 \pm 0.03$  mg ai L<sup>-1</sup> ( $n=4$ ),  $2.07 \pm 0.03$  mg ai L<sup>-1</sup> ( $n=6$ ), and  $4.29 \pm 0.02$  mg ai L<sup>-1</sup> ( $n=3$ ) for the nominal concentrations of 1, 2, and 4 mg ai L<sup>-1</sup>, respectively.

### *Dioecious hydrilla*

Control of dioecious hydrilla shoot biomass was similar for endothall 2 and 4 mg ai L<sup>-1</sup> treatments (Figure 3A). Control of 1 mg ai L<sup>-1</sup> with a 48-h exposure time (1/48) was  $13.3 \pm 11.1\%$ , while control of 1 mg ai L<sup>-1</sup> with a 96-h exposure time (1/96) was  $41.0 \pm 15.1\%$ . Shoots were still green and healthy with new growth present for both treatments. Although the 1/96 treatment provided partial control ( $<50\%$ ), plants from this treatment would probably recover based on visual observations.

Control for the other endothall treatments ranged from 74 to 99%. Plants treated with 4 mg ai L<sup>-1</sup> for a 48-h exposure time were killed without any remaining shoot biomass, or had remaining shoot biomass that was completely decayed without recovery potential. Control of this treatment matched that predicted by the CET curve (Netherland et al. 1991). Control for the other treatments (2/48, 2/72, and 4/24) was more variable and provided about 10% less control than that predicted by Netherland et al. (1991) and other studies (Pennington et al. 2001, Skogerboe and Getsinger 2001). These studies, however, used plants propagated from apical shoots and not tubers.

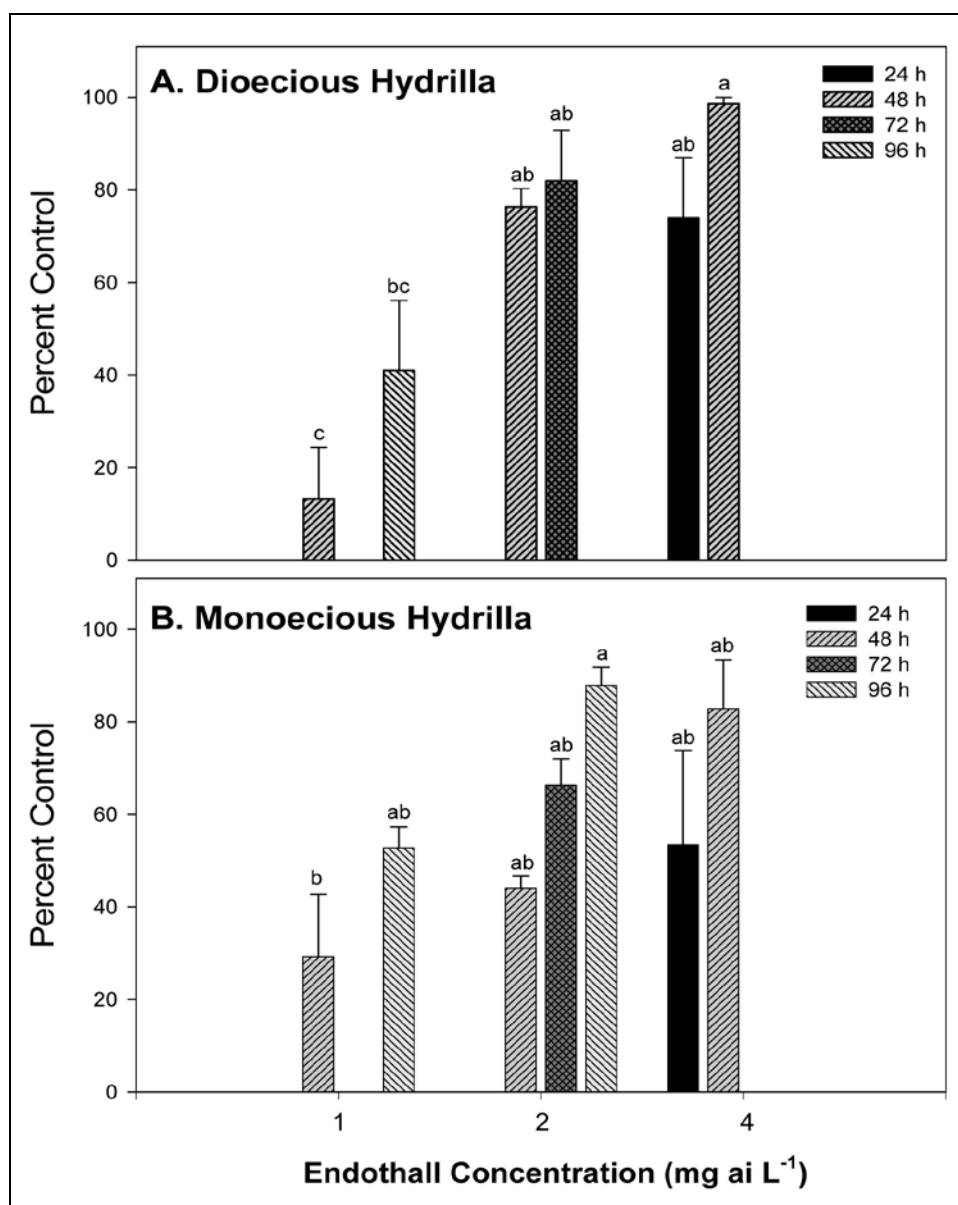


Figure 3. Percent control of A) dioecious and B) monoecious hydrilla propagated from tubers using 1, 2, and 4 mg ai L<sup>-1</sup> endothall under various exposure periods (24, 48, 72, and 96 h). Means are  $\pm 1$  SE ( $n=3$ ). Treatments with different letters are significantly different (S-N-K,  $p \leq 0.05$ ).

#### *Monoecious hydrilla*

Control of monoecious hydrilla shoot biomass was similar for all treatments, except the 1/48 which provided only  $29.2 \pm 13.5$  percent control (Figure 3B). Control of the other endothall treatments ranged from 53 to 88%. Endothall treatments that provided partial control (45 to 65%) were 1/96, 2/48, 2/72, and 4/24 treatments. Remaining shoot biomass from these treatments was green and healthy, suggesting recovery and regrowth



of treated plants. The 2/96 and 4/48 treatments provided  $82.9 \pm 10.5$  and  $87.9 \pm 3.89$  percent control, respectively. These treatments were successful because there were few viable stems present as a source of regrowth. Like the dioecious hydrilla in this study, control of monoecious hydrilla grown from tubers were less than that predicted by the endothall CET relationships generated for dioecious hydrilla by Netherland et al. (1991).

Results from this study show that endothall is efficacious against monoecious hydrilla. It provided good control with concentrations that were coupled with adequate exposure times (e.g., rates 2 to 4 mg ai L<sup>-1</sup> for 48 to 96 hours). Control of both dioecious and monoecious hydrilla grown from tubers was less than that predicted from previously documented endothall CET relationships developed for dioecious hydrilla. Refinement of endothall efficacy against both monoecious and dioecious hydrilla grown tubers is warranted.

## **Study 2. Monoecious Hydrilla Treated with Carfentrazone-ethyl and Flumioxazin**

**Objectives:** To determine whether pH affects the efficacy of the protoporphyrinogen oxidase (protox) inhibitors carfentrazone-ethyl and flumioxazin against monoecious hydrilla under short exposure times.

### **Materials and Methods**

This study was conducted in a controlled-environment growth chamber (58 m<sup>2</sup>) at the ERDC with an air temperature of  $24 \pm 2^\circ\text{C}$ , light intensity of  $462 \pm 24 \mu\text{mol m}^{-2} \text{sec}^{-1}$ , and photoperiod of 14 h:10 h light:dark cycle. Lighting was provided by a combination of 400 watt high-pressure sodium and metal halide bulbs. Experimental conditions within the chamber were maintained to simulate ambient conditions conducive for submersed plant growth.

Monoecious hydrilla tubers (mean tuber weight=  $0.11 \pm 0.01$  g FW,  $n=49$ ) were collected from North Carolina, transferred in a large tub filled with RO water, and sprouted in the growth chamber under ambient conditions over a two-week period. Three sprouted tubers (mean shoot length= $8.4 \pm 0.5$  cm) were planted in 300 ml glass beakers filled with sediment amended with 150 mg L<sup>-1</sup> ammonium chloride. A 1-cm layer of silica sand was added to the sediment surface to prevent suspension of sediment particles in the water column. Three beakers of monoecious hydrilla were placed in each of

24 vertical aquaria (10-L), which were filled with growth solution (Smart and Barko 1985). Plants grew for 38 d before herbicide application.

Pretreatment harvest was conducted one day before herbicide application for shoot biomass estimation. All shoots in one beaker from each aquarium were cut at the sediment surface and dried at 70 °C for 48 h to obtain g DW. Pretreatment shoot biomass was  $0.98 \pm 0.05$  g (mean  $\pm 1$  SE,  $n=24$ ), which represented a dense summer stand in North Carolina (Harlan et al. 1985). Since aqueous degradation of protox inhibitors are affected by pH, water column pH levels of 7 and 9 were used as treatments in this study. The pH of the growth solution ranges from 8 to 9 in aquaria with plants; therefore, additions of 0.1 M HCl were made as needed to lower pH in the pH 7 treatments 2.5 h before herbicide application. There were no additions of acid to the pH 9 treatment aquaria.

A 426 mg L<sup>-1</sup> stock solution of carfentrazone-ethyl (formulated as Stingray®, FMC Corp., Philadelphia, PA) calculated by percent ai was prepared. From the stock, 200 µg ai L<sup>-1</sup> of herbicide was applied to 24 aquaria for an exposure period of 6 h. A 510 mg L<sup>-1</sup> stock solution of flumioxazin (formulated as Payload®, Valent USA Corp., Walnut Creek, CA) calculated by percent ai was prepared. From this stock, 200 µg ai L<sup>-1</sup> of herbicide was applied to 24 aquaria for an exposure period of 6 h. An untreated reference (0 mg ai L<sup>-1</sup>) compared plant growth in the absence of herbicide dosage.

During herbicide exposure, water column pH was allowed to “drift” in all treatments. Measurements of pH and conductivity were made at the beginning and end of herbicide exposure with a multi-parameter probe (Model 556, YSI Instruments, Yellow Springs, OH). At the end of the 6-hour herbicide exposure period, all aquaria were drained and refilled with growth solution twice to remove all remaining aqueous herbicide residues.

Reapplication of carfentrazone-ethyl was made 21 days after initial treatment (DAIT), when a new stock solution was prepared as before, and a rate of 200 µg ai L<sup>-1</sup> was again applied to aquaria for a 6-hour exposure time. One hour before herbicide application, water column pH was adjusted to 7 with additions of 0.1 M HCl as needed in the pH 7 treatments. Treatments of pH 9 were not adjusted. During herbicide exposure, pH was allowed to “drift” in all treatments. Measurements of pH and conductivity were again made at the beginning and end of the herbicide exposure period. After 6 h,

all aquaria were drained and filled with growth solution twice to remove all herbicide residues. This study continued for another 21 days after the second herbicide treatment at which time plants were harvested (42 DAIT). Shoots from 2 beakers were clipped at the sediment surface, dried at 70 °C for 48 h to obtain DW. Water temperature was measured continuously with an Optic Stowaway® Temperature Probe in the reference aquaria.

There were four replicates for each treatment, including the reference. Shoot biomass data were analyzed using a two-way ANOVA to test for herbicide concentration and pH effects. If effects were significant ( $p \leq 0.05$ ), the S-N-K method was used as a means comparison procedure.

## Results and Discussion

Values for water temperature, pH, and conductivity indicated conditions that were conducive for aquatic plant growth under experimental conditions (Table 3; Smart and Barko 1985). Mean water temperature during this study was  $24.1 \pm 0.01$  °C. Water column pH did not vary widely between the reference and plants that were dosed with carfentrazone-ethyl. During the pH 7 treatments, the pH in the water column ranged from 6.9 to 7.1 at the beginning of herbicide exposure then drifted to a range of 9.2 to 9.5 at the end of the exposure period for both applications (Table 3). For the pH 9 treatments, the water column pH ranged from 9.7 to 10.2 during the 6-h exposure period for the first herbicide application, and from 8.6 to 9.0 for the second herbicide application.

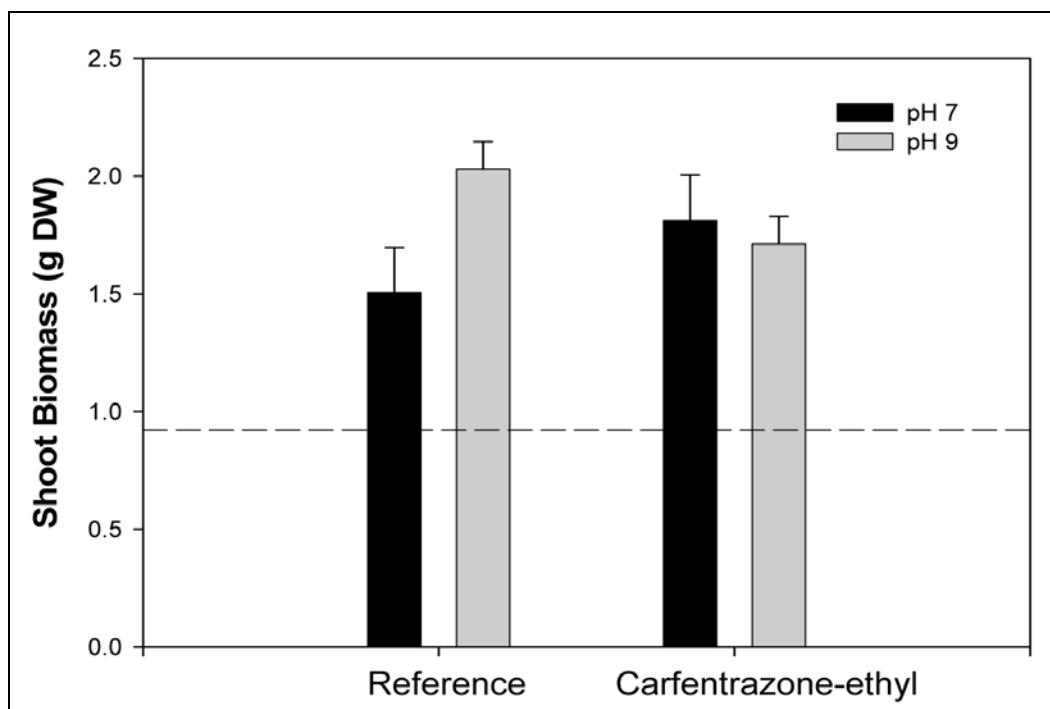
**Table 3. Range of mean water column pH and conductivity during initial and reapplication (second) of carfentrazone-ethyl and flumioxazin during a 6-h exposure period.**

Treatment	pH		Conductivity ( $\mu\text{S cm}^{-1}$ )	
	Initial	Second	Initial	Second
<b>Reference</b>				
pH 7	6.9 - 9.2	6.9 - 9.4	254 - 259	330 - 331
pH 9	9.7 - 10.2	8.9 - 9.0	280 - 294	363 - 364
<b>Carfentrazone-ethyl</b>				
pH 7	7.1 - 9.5	6.9 - 9.5	260 - 266	315 - 316
pH 9	9.8 - 10.2	8.6 - 8.9	345 - 358	325 - 325
<b>Flumioxazin</b>				
pH 7	6.9 - 9.3	7.0 - 9.4	257 - 260	326 - 328
pH 9	9.8-10.2	8.9 - 8.9	296-310	313 - 314

Water column pH was not a significant factor in shoot production of monoecious hydrilla dosed with carfentrazone-ethyl ( $p=0.207$ , Table 4). Plants dosed with carfentrazone-ethyl were comparable to the reference ( $p=0.963$ ; Table 4 and Figure 4). Herbicide symptoms were noted right after herbicide application, when plant apices and stems were chlorotic; however, plants were green and healthy by the end of the study.

**Table 4. Results of a two-way analysis of variance (ANOVA) for carfentrazone-ethyl treatments (rate= 0 and 200  $\mu\text{g ai L}^{-1}$  and pH= 7 and 9) on monoecious hydrilla shoot biomass.**

Source of Variation	DF	MS	F	P
Rate	1	0.001	0.002	0.963
pH	1	0.181	1.778	0.207
Rate x pH	1	0.388	3.814	0.075
Error	15	0.119		



**Figure 4. Monoecious hydrilla shoot biomass (g DW) after two applications of carfentrazone-ethyl (200  $\mu\text{g ai L}^{-1}$ ). The second application was made 21 days after initial treatment (DAIT) and biomass was harvested 42 DAIT. A 6-h exposure time was used in both applications, during which water column pH was either 7 or 9. Means are  $\pm 1$  SE ( $n=4$ ).**

Hydrolysis of carfentrazone-ethyl occurs quickly when water pH is above 7. Under laboratory conditions, the first order half-life of carfentrazone is 3.4 hr at pH 9 and 131 hours at pH 7 (Ngim and Crosby 2001). Although shoot biomass was not reduced in this study, further testing of

carfentrazone-ethyl should be conducted, particularly investigation of the interaction between pH, rate, and exposure time on carfentrazone-ethyl degradation. Moreover, studies that evaluate herbicide application to young plants would probably result in better control as plants in this study were mature and represented maximum summer biomass.

Water column pH was a significant factor in shoot production of monoecious hydrilla dosed with flumioxazin ( $p=0.019$ , Table 5). Plants significantly decreased compared to the reference ( $p=0.038$ ), where reductions in monoecious hydrilla shoot biomass with  $200 \mu\text{g ai L}^{-1}$  flumioxazin ranged from 12 to 23% (Figure 5). The suppression of monoecious hydrilla growth by flumioxazin was attributed to either pH or herbicide dosage, but not a combination of the two, since there were no significant interactions between herbicide rate and pH ( $p=0.341$ ).

Hydrolysis of flumioxazin occurs quickly when water pH is above 7. Under laboratory conditions, the half-life of flumioxazin is 14 to 22 min at pH 9 and 21 to 24 h at pH 7 (Payload® Herbicide Information Technical Bulletin, Valent USA Corp). That flumioxazin reduced shoot biomass at pH 9 suggests that, although hydrolysis of this product occurs in minutes, it is efficacious and higher rates with longer exposure times would provide better control. Moreover, herbicide application when plants are younger (before they reach maximum summer biomass that was simulated in this study) might also achieve better control. Further investigation of the interaction between pH, rate, and exposure time on flumioxazin degradation should be conducted.

**Table 5. Results of a two-way analysis of variance (ANOVA) for flumioxazin treatments (rate= 0 and  $200 \mu\text{g ai L}^{-1}$  and pH= 7 and 9) on monoecious hydrilla shoot biomass.**

Source of Variation	DF	MS	F	P
Rate	1	0.432	5.424	0.038
pH	1	0.589	7.390	0.019
Rate x pH	1	0.078	0.984	0.341
Error	15	0.137		

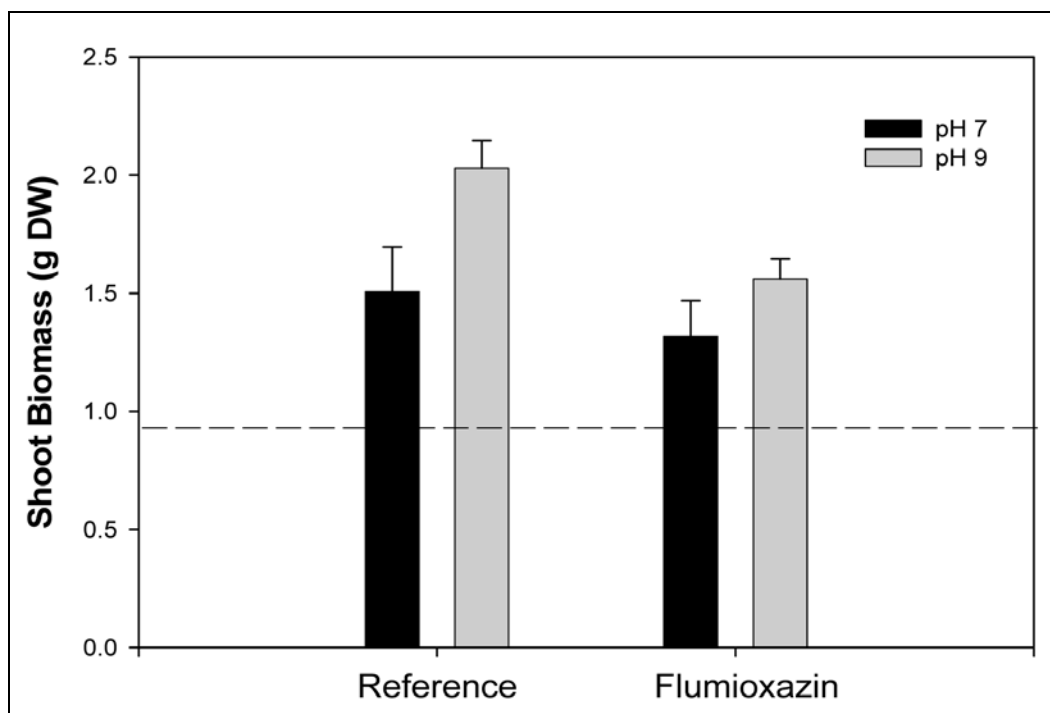


Figure 5. Monoecious hydrilla shoot biomass (g DW) after two applications of flumioxazin ( $200 \mu\text{g ai L}^{-1}$ ). The second application was made 21 d after initial treatment (DAIT) and biomass was harvested 42 DAIT. A 6-h exposure time was used in both applications, which water column pH was either 7 or 9. Means are  $\pm 1$  SE ( $n=4$ ).

### Study 3. Monoecious Hydrilla Treated with Penoxsulam

**Objective:** To determine low application rates of penoxsulam for controlling monoecious hydrilla under an extended exposure time.

#### Materials and Methods

This study was conducted in outdoor mesocosms at NCSU. Monoecious hydrilla shoots were collected from Lake Gaston, NC. Apical shoots were planted in hydrosol amended with nutrients in 45 x 18 cm flats and placed in 950-L concrete vaults modified for aquatic plant experimentation.

For herbicide applications, stock solutions of penoxsulam (formulated as Galleon®, SePRO Corp., Carmel, IN) and endothall (formulated as Aquathol® K) were prepared. Using these stock solutions, penoxsulam treatments included a 0 (untreated reference); 5, 10 and 20  $\mu\text{g/L}$  with a 90-d exposure time (DET); 5  $\mu\text{g/L}$  with a static exposure period; 20  $\mu\text{g/L}$  with 45 DET, and 10  $\mu\text{g/L}$  followed by (fb) endothall (2 mg/L) at 30 DAT (Table 6). The concentration of treatment 5 was maintained by flushing

the tank at 30 and 60 DAT and retreating. Each treatment was replicated three times and initial treatments were applied on 28 June 2006.

Plant control was visually rated weekly on a 0 to 100% scale with 0% equal to no control and 100% equal to complete plant death. Plant height was measured at approximately 42 DAT and 90 DAT. Plant shoots were harvested at 90 DAT for DW determination. After harvest, flats were placed back into vaults with fresh water and allowed to regrow. Regrowth was harvested at 42 d after initial harvest for dry weight determination. Hydrosol was sifted and tubers were collected and counted. All data was subjected to analysis of variance and Fisher's Protected LSD was used for mean separation.

## Results and Discussion

Hydrilla control generally increased across all treatments during the first month after penoxsulam applications. At 35 DAT, hydrilla control ranged from 50 to 93% with most penoxsulam treatments. Hydrilla height at 35 DAT was 10 to 14 cm with both 20 ug/L penoxsulam treatments. Height of the untreated control was greater (19 cm) and other treatments were generally similar to the untreated control. At 90 DAT, hydrilla was controlled 97 to 99% with 20 ug/L penoxsulam (90 DET) and penoxsulam fb endothall. Other treatments controlled hydrilla 42 to 72%. Hydrilla height at 90 DAT was lowest with penoxsulam fb endothall at 15 cm and highest with the untreated at 82 cm.

Initial DWs of hydrilla reflected excellent control with the 20 ug/L penoxsulam (90 DET) and penoxsulam fb endothall applications. Dry weight with the 20 ug/L penoxsulam treatment was only 7% of the untreated DW, whereas virtually no hydrilla was present for harvest with penoxsulam fb endothall. The DW of hydrilla regrowth after initial harvest was reduced from the untreated by all treatments except the two 5 ug/L penoxsulam treatments. Hydrilla tuber counts further reflected excellent control with 20 ug/L penoxsulam (90 DET) and penoxsulam fb endothall as less than 5 tubers were present with these treatments while 375 tubers were present in the untreated.

In summary, penoxsulam at 20 ug/L with 90 DET was adequate for hydrilla control in this trial. However, the minimum effective penoxsulam rate may be lower when a contact herbicide is applied following initial penoxsulam application, as was seen in this trial with penoxsulam fb

Table 6. Monoecious hydrilla response to selected penoxsulam treatments.

Pest Name				Hydrilla	Hydrilla	Hydrilla	Hydrilla	Hydrilla	Hydrilla	Hydrilla	Hydrilla	Hydrilla
Description												
Rating Date				7/5/2006	7/11/2006	7/19/2006	7/26/2006	8/4/2006	8/4/2006	8/8/2006	8/9/2006	8/25/2006
Rating Data Type				Control	Control	Control	Control	Control	Height	Control	Control	Control
Rating Unit				0-100%	0-100%	0-100%	0-100%	0-100%	cm	0-100%	0-100%	0-100%
Trt No.	Treatment Name	Rate	Rate Unit									
1	Nontreated Control			0	0	0	0	0	19	0	0	0
2	Penoxsulam*Galleon	20	ppb a	22	77	86	90	93	10	92	95	98
3	Penoxsulam*Galleon	10	ppb a	0	2	8	22	53	16	32	70	68
4	Penoxsulam*Galleon	5	ppb a	0	18	23	27	50	17	43	57	67
5	Penoxsulam*Galleon	20	ppb a	27	32	44	54	65	14	63	70	72
6	Penoxsulam*Galleon	5	ppb a	0	3	8	23	35	17	0	35	53
7	Penoxsulam*Galleon	10	ppb a	2	15	37	40	63	15	60	70	93
	Aquathol K*Endothall	2	ppm a									
LSD (P=.05)				31.6	38.6	44.5	51.8	47.8	3.4	59.7	43.7	37.7
Standard Deviation				18.1	22.0	25.4	29.6	27.3	2.0	34.1	24.9	21.5
CV				216.68	94.5	80.49	77.03	50.82	12.85	80.95	42.48	34.91



<b>Pest Name</b>				Hydrilla	Hydrilla	Hydrilla	Hydrilla	Hydrilla				
<b>Description</b>								Regrowth				
<b>Rating Date</b>				9/15/2006	9/15/2006	9/15/2006	11/2/2006	11/2/2006				
<b>Rating Data Type</b>				Control	Dry Weight	Height	Tuber	Dry Weight				
<b>Rating Unit</b>				0-100%	g	inch	count	g				
<b>Trt No.</b>	<b>Treatment Name</b>	<b>Rate</b>	<b>Rate Unit</b>									
1	Nontreated Control			0	166.6	32.0	375.7	31.2				
<b>Trt No.</b>	<b>Treatment Name</b>	<b>Rate</b>	<b>Rate Unit</b>									
2	Penoxsulam*Galleon	20	ppb a	99	11.3	10.5	4.0	0.8				
3	Penoxsulam*Galleon	10	ppb a	68	84.4	18.5	101.0	5.1				
4	Penoxsulam*Galleon	5	ppb a	67	70.0	29.5	195.7	14.7				
5	Penoxsulam*Galleon	20	ppb a	72	80.3	15.2	134.3	0.0				
6	Penoxsulam*Galleon	5	ppb a	53	111.0	18.4	282.0	13.2				
7	Penoxsulam*Galleon	10	ppb a	97	0.2	6.0	2.0	0.0				
	Aquathol K*Endothall	2	ppm a									
LSD (P=.05)				38.0	97.10	22.65	226.30	18.88				
Standard Deviation				21.7	55.44	12.94	129.21	10.78				
CV				34.84	70.3	64.11	77.84	123.81				

endothall. This is possibly due to burn-down of hydrilla foliage by the contact herbicide coupled with regrowth suppression by penoxsulam that had been translocated to roots. Penoxsulam at 10 ug/L or lower applied alone was generally inadequate for hydrilla control regardless of exposure time. Additional research is needed to confirm the findings from this study prior to issuing any guidance about the use of penoxsulam to control monoecious hydrilla on Lake Gaston.

#### **Study 4. Monoecious Hydrilla Treated with Imazamox**

**Objective:** To determine effective applications of imazamox for controlling monoecious hydrilla under an extended exposure time.

##### **Materials and Methods**

Monoecious hydrilla was collected from Lake Gaston, NC, and planted a green house in 15-cm deep pots containing a sandy loam soil typical of the region. Planted pots were placed in 73 L tubs containing 70 L of pond water. Each tub contained one pot of hydrilla with an average shoot height of 30 cm. An imazamox stock solution (formulated as Clearcast®, BASF Corp., Research Triangle Park, NC) calculated by percent ai was prepared. From this stock solution, in-water treatments were applied using a pipette and included imazamox at 0, 25, 50, 75, 100 and 200 ug/L and a sequential application of imazamox at 25 ug/L repeated every 4 wk. Each treatment was replicated four times. Plant control was visually rated on a 0 to 100% scale with 0% equal to no control and 100% equal to complete plant death. Ratings were taken monthly for four months. Plant height was also measured and plants were harvested for DW determination. After initial harvest, a second harvest was conducted four weeks later to measure plant regrowth. All data was subjected to analysis of variance and means separated according to Fisher's Projected LSD ( $P < 0.05$ ).

##### **Results and Discussion**

At approximately 1 month after treatment (MAT), hydrilla control did not exceed 70%, but was 78 to 96% at 3 MAT across imazamox rates (Table 7). Plant heights taken at 1.5 MAT were 8.7 to 10.3 cm across imazamox treatments and were 27 cm with the untreated reference (control). Untreated hydrilla dry weight was 39 g and greater than all treatments. Imazamox treatment of 200 ug/L resulted in hydrilla dry weight of 1.3 g. Other imazamox treatments resulted in hydrilla dry weight of 6.1 to 13.7 g.

Table 7. Monoecious hydrilla response to selected imazamox treatments.

Pest Type										
Pest Code										
Pest Name				Hydrilla	Hydrilla	Hydrilla	Hydrilla	Hydrilla	Hydrilla	Hydrilla
Part Rated										Regrowth
Rating Date				7/21/2006	8/3/2006	8/9/2006	9/12/2006	9/13/2006	9/12/2006	10/12/2006
Rating Data Type				Control	Height	Control	Control	Height	Dry Weight	Dry Weight
Rating Unit				0-100%	in	0-100%	0-100%	inch	g	g
Trt No.	Treatment Name	Rate	Rate Unit							
1	Nontreated Control			0	26.67	0	0	25.40	39.00	3.00
2	Imazamox*Clearcast	25	ppb a	25	8.67	69	78	12.50	11.62	3.03
3	Imazamox*Clearcast	50	ppb a	43	9.67	74	82	17.17	9.12	0.77
4	Imazamox*Clearcast	75	ppb a	33	10.33	64	77	14.13	13.68	0.57
5	Imazamox*Clearcast	100	ppb a	52	8.67	77	83	10.00	6.10	0.90
6	Imazamox*Clearcast	200	ppb a	70	7.67	88	96	5.00	1.31	0.50
7	Imazamox*Clearcast	25	ppb a	30	10.33	53	82	13.43	13.16	0.13
	Imazamox*Clearcast	25	ppb a							
	Imazamox*Clearcast	25	ppb a							
	Imazamox*Clearcast	25	ppb a							
LSD (P=.05)				24.6	2.166	20.7	12.4	12.186	18.774	2.379
Standard Deviation				14.0	1.237	11.7	7.1	6.909	10.552	1.358
CV				35.66	11.12	18.04	9.48	55.86	89.73	122.1

Some hydrilla regrowth was present with DW from 0.1 to 0.8 g with all imazamox rates, except at 25 ug/L. In summary, imazamox provided good control of monoecious hydrilla, particularly at the highest rate tested. Lower imazamox rates generally resulted in less control. Multiple applications of 25 ug/L imazamox did not provide better control, height reduction, or dry weight reduction than a single 25 ug/L imazamox application, except after initial harvest. Additional evaluations are needed to confirm the observations noted above, and to evaluate the potential utility of combining low rate imazamox applications with contact herbicides.

*Eurasian watermilfoil – Nuisance Exotic Species.*

Summarized results from previous studies using carfentrazone-ethyl, diquat, endothall, fluridone, triclopyr, and 2, 4-D are provided below:

**Carfentrazone-ethyl.** This product is a contact-type herbicide registered in the U.S. for aquatic sites in 2004 (Netherland et al. 2005). It rapidly hydrolyzes when water pH > 7, and as such, exposure time for effective Eurasian watermilfoil control may be less than 24 h. Carfentrazone-ethyl provided partial control of Eurasian watermilfoil (50 to 70%) in an outdoor mesocosm study with a water pH of 9 (Glomski et al. 2006). In this study, carfentrazone-ethyl provided >90% control under static exposure conditions at the maximum label rate (200 ug ai/L) and with a water column pH of 8. Gray et al. (2007), showed  $\geq 98\%$  control of Eurasian watermilfoil under static exposure at application rates of  $\geq 150$  ug ai/L. Because carfentrazone-ethyl is a contact herbicide, Eurasian watermilfoil regrowth would be expected after one season of control. Carfentrazone-ethyl is considered a selective herbicide (Thompson and Nissen 2000) in terrestrial agriculture, and has the potential for selective control of Eurasian watermilfoil in aquatic systems (Glomski et al. 2006). While carfentrazone-ethyl shows good activity and control on Eurasian watermilfoil, more refinement of CET relationships and lake studies are required for a better understanding of the product's performance in the field.

**Diquat.** Diquat (formulated as Reward®) is a contact-type herbicide that provides a rapid kill of submersed plant shoots, followed by a quick decomposition of the affected tissue (within 4 to 7 d post-treatment). The application window for optimum plant control is in late spring when target vegetation is actively growing and water temperature is above 12 °C. Extensive treatment experience has shown that one application of diquat at recommended rates can provide greater than 80% knockdown of Eurasian

watermilfoil plants, with regrowth occurring during 6 to 8 wk post-treatment. Since it is a broad-spectrum product, actively growing shoots of non-target native plants that occur within the treated zone will also be controlled. Because diquat is readily and strongly bound to mineral clays and organic matter, this herbicide is most effective when used in clear water. Use of diquat in turbid water conditions will inactivate the product and result in poor or no control of treated vegetation (Hofstra et al. 2001; Poovey and Getsinger 2002). Currently, there are no established CET relationships for diquat to allow for its use as a method for selectively controlling Eurasian watermilfoil. When used at rates effective for controlling that target plant, diquat will also control other native plants actively growing in the treated zone. Consequently, the most appropriate use of diquat for Eurasian watermilfoil control in Lake Gaston would be for relatively small-scale, partial lake applications, where broad spectrum removal of most submersed plants in those settings would only represent a small proportion of the total lake-wide plant community. Diquat is available as a liquid formulation.

**Endothall.** Endothall is a contact-type herbicide that has been used in the U.S. for aquatic weed control for nearly 50 yr (Netherland et al. 2005). Development of endothall (formulated as Aquathol® K, United Phosphorus International, King of Prussia, PA) CET relationships indicate that Eurasian watermilfoil injury is directly proportional to the length of time plants are in contact with a given endothall concentration (Netherland et al. 1991). At recommended treatment rates, exposure time should be maintained for at least 18 to 24 h for best results (Netherland et al. 1991). Given these exposure times, water in treatment areas should be quiescent with minimal flow, and applications should be made in areas > 2 ha (5 acres) in size. When used in this manner, there is a rapid kill of plant shoots that results in >80% knockdown within a year of treatment; however, re-growth can occur in 6 to 8 wk. Herbicide applications should be made in spring when water temperatures are above 12 °C and plants are actively growing. Endothall is not affected by water turbidity (Hofstra et al. 2001) and can provide plant control in areas protected from high water exchange processes, such as coves, and boat marinas. Endothall is generally considered a non-selective herbicide and recommended application rates may impact some native submersed vegetation. However, small-scale studies have shown that low rates of endothall applied in early spring with exposure times of 1 to 3 d, can be efficacious against Eurasian watermilfoil with minimal damage to non-target vegetation (Skogerboe and Getsinger 2001, Skogerboe and Getsinger

2002, Skogerboe and Getsinger 2006). Endothall is available as liquid and granular formulations of a dipotassium salt or an alkyl amine.

**Fluridone.** This systemic product (formulated as Sonar® AS and AVASAT!®, SePRO Corp., Carmel, IN) has been used for submersed weed control in the US since 1986 (Netherland et al. 2005). It is a slow-acting, low-dose, long-contact-time herbicide. Once fluridone is absorbed by the plant leaves and stems, it interrupts the carotenoid biosynthetic pathway; carotenoid pigments are necessary for plants to photosynthesize. It requires a 45- to 60-d exposure time to be effective. Susceptible plants die and decompose slowly, with >90% knockdown in year of treatment. If the treatment is effective, target plant regrowth usually does not occur for over 12 months (Netherland et al. 1993, Netherland and Getsinger 1995a, 1995b). Low rates are selective for Eurasian watermilfoil, with minimal injury to non-target plant species (Netherland et al. 1997, Getsinger et al. 2002). Fluridone efficacy is best provided with whole lake treatments, or very large treatment blocks,  $\geq 40$  ha (100 acres) in large water bodies where water exchange processes are limited. Fluridone is formulated as a liquid that is applied in the form of an aqueous suspension, or as a granular material.

**Triclopyr.** Triclopyr (formulated as Renovate®, SePRO Corp., Carmel, IN) is similar to 2,4-D in its mode of action and translocation, and this systemic auxin is effective against Eurasian watermilfoil requiring exposure times of 1 to 3 d (Netherland and Getsinger 1992; Petty et al. 1998). It has been available for use in the U.S. in aquatic sites since 2001 (Netherland et al. 2005). Stem epinasty and browning occurs 1 to 2 d after application, while plant decomposition occurs 14 to 28 d after application. Triclopyr is most efficacious against young, actively growing plants. Eurasian watermilfoil may be controlled for three years, including the year of treatment, with no adverse impacts to native vegetation (Getsinger et al. 1997). Nonetheless, plant regrowth may occur in 4 to 6 wk if Eurasian watermilfoil is not completely killed during herbicide application (Poovey et al. 2004). Formulations include liquid and granular amines.

**2,4-D.** This herbicide is classified as systemic and acts as an auxin-like plant hormone and has been used in the U.S. in aquatic sites for over 60 yr (Netherland et al. 2005). Once absorbed into plant tissues, there is a moderately slow kill of shoots (7 to 14 d) and decomposition of plants (14 to 28 d), with >85% knockdown of mature shoots within the year of treatment. Young, actively growing milfoil plants are more susceptible to 2,4-D than

are mature, slowly growing plants. In cases where milfoil is not completely killed, regrowth can occur in eight to twelve weeks following the initial application. Control of Eurasian watermilfoil is selective at all rates, with no, or minimal, injury to most non-target plants, particularly the monocots - grass family (Getsinger et al. 1982, Parsons et al. 2001, Getsinger et al. 2002, Skogerboe and Getsinger 2006). Partial lake treatments using 2,4-D would include moderately-sized blocks (2 – 4 ha, or 5 to 10 acres) or all areas in the littoral zone infested with Eurasian watermilfoil. Formulations include a liquid dimethyl amine (DMA) and a granular clay butoxyethanol ester (BEE).

**Current Evaluations to Determine CET Information.** Herbicides CETs evaluated in this section (Studies 5 through 9) include the contact herbicides, carfentrazone-ethyl and flumioxazin, and the systemic herbicides, bispyribac-sodium, imazamox, and penoxsulam.

## **Study 5. Eurasian Watermilfoil Treated with Carfentrazone-ethyl**

**Objective:** To evaluate efficacy of carfentrazone-ethyl against Eurasian watermilfoil under a static herbicide exposure period.

### **Materials and Methods**

This pilot study was conducted at the ERDC in Vicksburg, MS, in a controlled-environment growth chamber (58 m<sup>2</sup>). Experimental conditions within the chamber were maintained to mimic ambient conditions conducive for submersed plant growth: water temperature of 24 ± 1° C, light intensity of 300 ± 80 µmol/m<sup>2</sup>/sec, and a photoperiod of 14:10-h light:dark cycle. Eurasian watermilfoil was obtained from culture tanks at the ERDC from samples originally collected in Wisconsin. Eurasian watermilfoil was planted by placing five apical tips (15 cm length) in each beaker (750 ml), filled with sediment collected from a nearby lake and amended with ammonium chloride at a rate of 0.2 g/L. A 1-cm layer of silica sand was added to the sediment surface to prevent suspension of sediment particles in the water column. Four planted beakers were then placed in six vertical aquaria (50 L) filled with 48 L of culture solution (Smart and Barko 1985). Plants were allowed to establish under these conditions for approximately 3 wk prior to treatment. During the plant establishment period, pH of the water was measured every 2 to 3 d in the morning and afternoon, since carfentrazone-ethyl degradation is sensitive to aqueous pH. At time of treatment, mean pH of the water was 8.4 ± 0.7.

A 426 mg L<sup>-1</sup> stock solution of carfentrazone-ethyl (formulated as Stingray®) calculated by percent ai was prepared. From this stock, 200 µg ai L<sup>-1</sup> of carfentrazone-ethyl was applied to each aquaria and a two week exposure time was imposed. Untreated reference aquaria were included in the study to evaluate plant growth in absence of the herbicide.

After two weeks, all aquaria were drained and refilled three times with culture solution to remove aqueous herbicide residues. Post-treatment biomass measurements were made by harvesting one beaker 2 and 4 wk after treatment (WAT) and the two remaining beakers 6 WAT. After each harvest, all shoot material was dried at 70 °C for 48 h to obtain a g DW biomass measurement for each replicate. Carfentrazone-ethyl treatments were assigned to aquaria in a completely randomized manner and replicated four times (references were only replicated twice). Biomass data are reported as means ± standard error (SE).

## Results and Discussion

At five days after treatment, herbicide symptoms included loss of turgor, some color loss, shoot and leave deterioration, and growth inhibition. During the final harvest (6 WAT), treated shoots were brown and decaying, but some green coloration observed at the base of a few shoots (three to five). Very limited regrowth of shoots was noted.

A considerable reduction in shoot biomass of carfentrazone-ethyl treated Eurasian watermilfoil was measured in this study (Figure 6). Plants treated at 200 µg ai/L showed a biomass reduction of 80% at 2 WAT, 74% at 4 WAT, and 79% at 6 WAT. Untreated references remained green and healthy, and continued growing throughout the study period.

In future evaluations, a rate range of carfentrazone-ethyl with short exposure times, in combination with a shoot regrowth component, should be investigated. This information will be necessary prior to developing guidance for using carfentrazone-ethyl to control Eurasian watermilfoil in Lake Gaston.



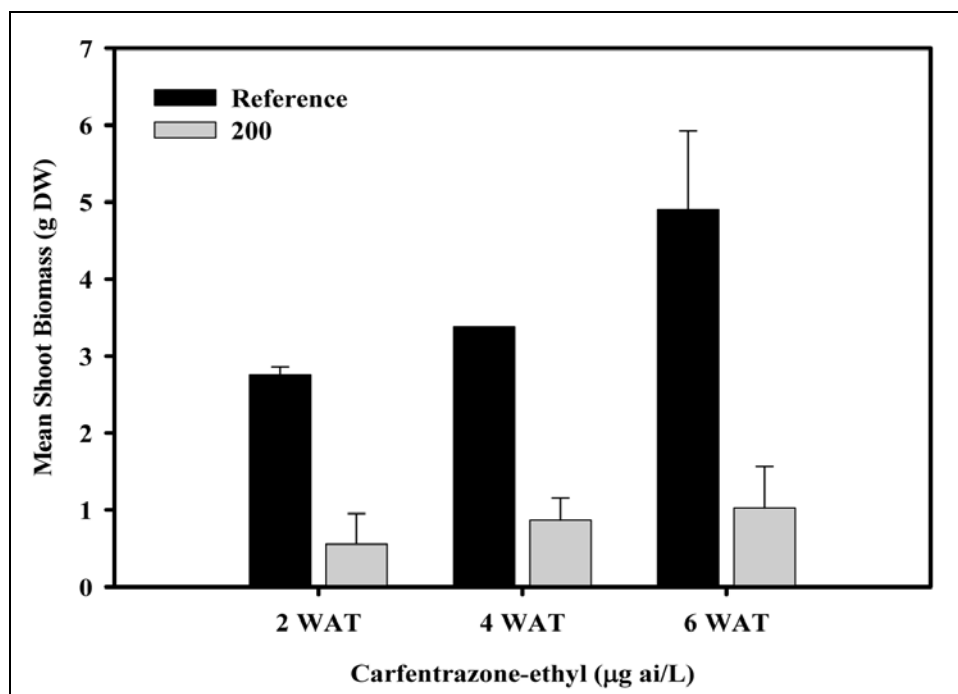


Figure 6. Mean dry weight shoot biomass (g DW) of Eurasian watermilfoil 2, 4, and 6 wk after treatment (WAT) to a 2-wk exposure of carfentrazone-ethyl. Vertical bars represent the mean  $\pm$  SE of four replicates (reference two replicates).

## Study 6. Eurasian Watermilfoil Treated with Flumioxazin

**Objective:** To evaluate efficacy of flumioxazin against Eurasian watermilfoil under a static exposure period.

### Materials and Methods

This pilot study was conducted at the ERDC in Vicksburg, MS, in a controlled-environment growth chamber under the same conditions as reported in Study 5, above. Eurasian watermilfoil was collected from culture tanks at ERDC from samples originally collected in Wisconsin. Eurasian watermilfoil was planted and established under the same conditions reported in Study 5, above.

A 510 mg L<sup>-1</sup> stock solution of flumioxazin (formulated as Payload®) calculated by percent ai was prepared. From this stock, 200 µg ai L<sup>-1</sup> of flumioxazin was applied to each aquaria and a 2-wk exposure time was imposed. Untreated reference aquaria were included in the study to evaluate plant growth in absence of the herbicide.

After two weeks, all aquaria were drained and refilled three times with culture solution to remove aqueous herbicide residues. Post-treatment biomass measurements were made by harvesting one beaker 2 and 4 WAT and the two remaining beakers 6 WAT. After each harvest, all shoot material was dried at 70 °C for 48 h to obtain a g DW biomass measurement for each replicate. Flumioxazin treatments were assigned to aquaria in a completely randomized manner and replicated four times (references were only replicated twice). Biomass data are reported as means  $\pm$  SE.

## Results and Discussion

As early as three days after treatment, plants were discolored, exhibited turgor loss and shoots began to collapse and sink. By 1 WAT, all plants were brown and partially collapsed to the bottom. During the final harvest, only a few small (15 cm-long) leafless shoots remained. A considerable reduction in shoot biomass of flumioxazin-treated Eurasian watermilfoil was measured in this study (Figure 7). Plants treated with flumioxazin at 200  $\mu\text{g ai/L}$  showed biomass reduction of 95% at 2 WAT, 92% at 4 WAT, and 97% at 6 WAT. Untreated references remained green and continued growing throughout the study.

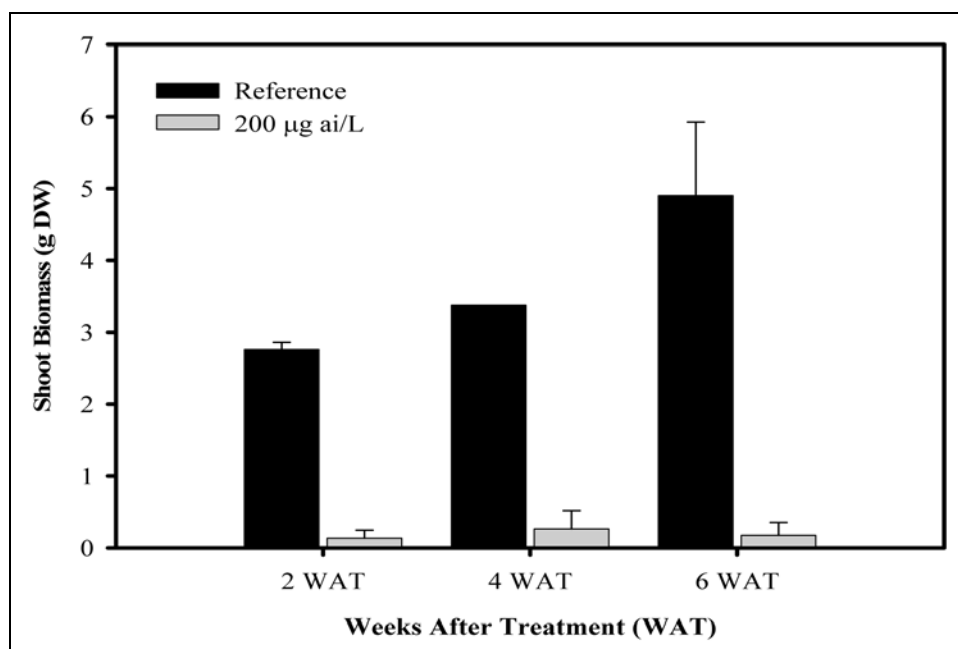


Figure 7. Mean dry weight shoot biomass (g DW) of Eurasian watermilfoil 2, 4, and 6 wk after treatment to a 2-wk exposure of flumioxazin. Vertical bars represent the mean  $\pm$  SE of four replicates (reference two replicates).

In future evaluations, a rate range of flumioxazin with short exposure times, in combination with a shoot regrowth component, should be investigated. This information will be necessary prior to developing guidance for using flumioxazin to control Eurasian watermilfoil in Lake Gaston.

## **Study 7. Eurasian Watermilfoil Treated with Bispyribac-sodium**

**Objective:** To evaluate the efficacy of bispyribac-sodium against Eurasian watermilfoil under a long herbicide exposure time regime.

### **Materials and Methods**

This study was conducted at the ERDC in a controlled-environment growth chamber (58 m<sup>2</sup>). Experimental conditions within the chamber were maintained to mimic ambient conditions conducive for submersed plant growth: water temperature of 24 ± 1 °C, light intensity of 300 ± 80 µmol/m<sup>2</sup>/sec, and a photoperiod of 14:10-h light:dark cycle.

Eurasian watermilfoil was collected from outdoor ponds located at the LAERF. Three apical tips (15 cm length) were planted in each beaker (300 ml), filled with sediment collected from a nearby lake and amended with ammonium chloride at a rate of 0.2 g/L and Osmocote® (18-6-12) at a rate of 4.0 g/L. A 1-cm layer of silica sand was added to the sediment surface to prevent suspension of sediment particles in the water column. Six planted beakers were placed in 28 vertical aquaria (50 L) and filled with 48 L of culture solution (Smart and Barko 1985). Plants were allowed to establish under these conditions for approximately 3 wk prior to treatment. At time of treatment, plants were healthy and actively growing.

A stock solution of bispyribac-sodium (formulated as Velocity®, Valent USA Corp., Walnut Creek, CA), calculated by percent ai was prepared. From this stock, six rates of bispyribac-sodium (2.5, 5, 10, 20, 40, and 80 µg ai/L) were applied to aquaria and a static exposure was imposed. Untreated reference aquaria were included to evaluate plant growth in absence of herbicide.

One day prior to treatment, a harvest of all shoot biomass from two beakers was performed. Post-treatment biomass measurements were made by harvesting two additional beakers 6 and 11 WAT. After each

harvest, all shoot material was dried at 70 °C for 48 h to obtain a g DW biomass measurement for each replicate.

Bispyribac-sodium treatments were assigned to aquaria in a completely randomized manner and replicated four times. Means for each replicate were calculated from post-treatment harvest data, and then subjected to a one-way ANOVA using Sigmastat (version 3.1, Systat Software, Inc., Point Richmond, CA). If the assumptions of normality and equal variance were not met, data was analyzed using the Kruskal-Wallis one-way ANOVA based on ranks. If effects were significant ( $p \leq 0.05$ ), means were separated using the S-N-K method.

## Results and Discussion

At 2 WAT, all herbicide treated plants had visual injury symptoms, except plants treated at 2.5 µg ai/L. At this time, plants treated at 5 and 10 µg ai/L were mostly green with slight browning and epinasty, while plants treated with rates of  $\geq 20$  µg ai/L displayed epinasty, chlorosis, browning of shoots and leaves, and growth inhibition. At 6 WAT, plants treated at 2.5 and 5 µg ai/L were still green with some browning and growth inhibition, while plants treated with bispyribac-sodium at  $\geq 10$  µg ai/L were brownish-black in color, epinastic with loss of turgor, and collapsing. During the final harvest at 11 WAT, plants treated at 2.5 and 5 µg ai/L still remained green with some yellowing. Plants treated at  $\geq 10$  µg ai/L remained brownish-black in color and epinastic, with most collapsed and deteriorating. Regrowth (i.e., shoots ~ 15 cm in length) was observed in all replicates of 2.5, 5, and 10 µg ai/L treatments at 11 WAT, but these new shoots still showed injury symptoms (i.e., epinasty, slight yellowish-browning).

Eurasian watermilfoil shoot biomass was significantly less than the untreated reference in bispyribac-sodium treatments  $\geq 10$  µg ai/L at 6 WAT, and this trend continued through the termination of the study (Figure 8). Shoot biomass reduction compared to the untreated reference ranged from 0% to 94% at 6 WAT and 5% to 99% at 11 WAT (Figure 8). Typically, a successful herbicide treatment in the field is considered as reducing shoot biomass by 90% or greater. In this study, shoot biomass was reduced by 94% at 80 µg ai/L, 6 WAT, and by 98-99% at 20, 40, and 80 µg ai/L, 11 WAT.

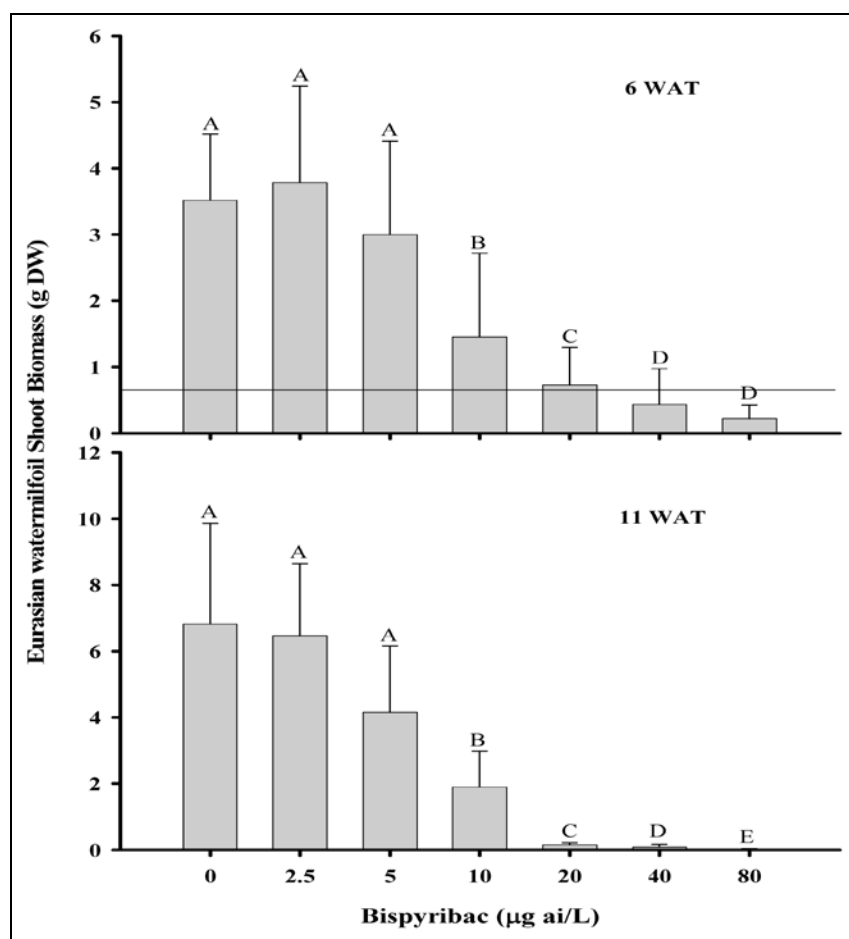


Figure 8. Mean shoot biomass (g DW) of Eurasian watermilfoil 6 and 11 wk after treatment (WAT) to a static exposure of bispyribac-sodium. Vertical bars represent the mean of four replicates. The horizontal line at 6 wk after treatment represents mean pretreatment shoot biomass. Letters indicate significant treatment differences according to the Student-Newman-Keuls (S-N-K) method ( $p \leq 0.05$ ).

Results indicate that bispyribac-sodium is efficacious against Eurasian watermilfoil and application rates of  $\geq 20$  µg ai/L are required to provide  $\geq 90\%$  control of that target plant for extended exposure periods (11 WAT). However, refinement of CET relationships (e.g. post-treatment plant recovery data, application rates vs. shorter exposure times, impacts on non-target plants) are required prior to developing guidance for operational use on Lake Gaston.

### Study 8 (A, B, and C). Eurasian Watermilfoil treated with Imazamox

**Objective:** To evaluate efficacy of imazamox against Eurasian watermilfoil under a long herbicide exposure time regime.

## Study 8A

### Materials and Methods

This study was conducted at the ERDC in a controlled-environment growth chamber (58 m<sup>2</sup>). Within the chamber, 20 vertical aquaria (50 L) were used to evaluate the herbicide imazamox against Eurasian water-milfoil. Conditions within the growth chamber were maintained to mimic ambient conditions conducive for submersed plant growth : water temperature  $24 \pm 1$  °C with a light intensity of  $311 \pm 75$  µmol/m<sup>2</sup>/sec and a 10:14-h light:dark photoperiod.

Eurasian watermilfoil was collected from Medicine Lake, Minnesota. After rinsing all plant material, three apical stems (15 cm length) were planted in each beaker (450 ml) filled with sediment collected from a nearby lake and amended with ammonium chloride at a rate of 0.2 g/L. A thin layer (0.5 cm) of silica sand was then added to the sediment surface to reduce sediment and nutrient dispersion into the water column. Five planted beakers were placed in each of 20 aquaria (50-L) and filled with 48 L of culture solution (Smart and Barko 1985). To maximize herbicide uptake, plants were treated before canopy formation three weeks after planting when shoots had just reached the water's surface. At the time of treatment, plants were green, healthy and actively growing.

Prior to herbicide treatment, one beaker was harvested from each aquarium to obtain a shoot length and biomass pretreatment measurement. Shoot length was measured by cutting all plant material from the surface of the sediment within the beaker and all shoots were measured to the nearest centimeter. All plant material from each beaker was then oven-dried at 70°C for 48 h and weighed to the nearest g DW.

An imazamox stock solution (formulated as Clearcast®) was prepared. From this stock solution, imazamox rates of 2.5, 5, 10, and 20 µg ae/L were applied to treatment aquaria. An additional treatment, which served as a reference with no herbicide (0 µg ae/L), was also present. At 45 DAT, the four remaining beakers in the aquaria were removed, and the plants were harvested. Total shoot length was recorded and plants were dried at 70 °C for 48 h to obtain biomass measurements. Shoot length and shoot biomass are reported as the sum of four beakers total length (cm) and the sum of four beakers (g DW), respectively.

Each treatment was replicated four times. If shoot length or biomass data did not meet the assumptions of normality or equal variance, data was square root transformed, then reanalyzed using one-way ANOVA to determine differences in herbicide concentrations ( $p \leq 0.05$ ). If significant treatments were detected, the Holm-Sidak means comparison procedure was performed.

## Results and Discussion

No visual symptoms of herbicide injury were apparent in any imazamox treatments throughout the study. None of the imazamox rates evaluated in this study significantly reduced Eurasian watermilfoil shoot length (Figure 9) or shoot biomass (Figure 10) compared to the untreated reference. Additionally, no rate of imazamox reduced Eurasian watermilfoil shoot length greater than 10% (range 2-10%), and 3 of the 4 rates evaluated had higher biomass values than the untreated reference. Results from this study indicate that higher application rates of imazamox should be evaluated to determine whether the product has any activity on Eurasian watermilfoil.

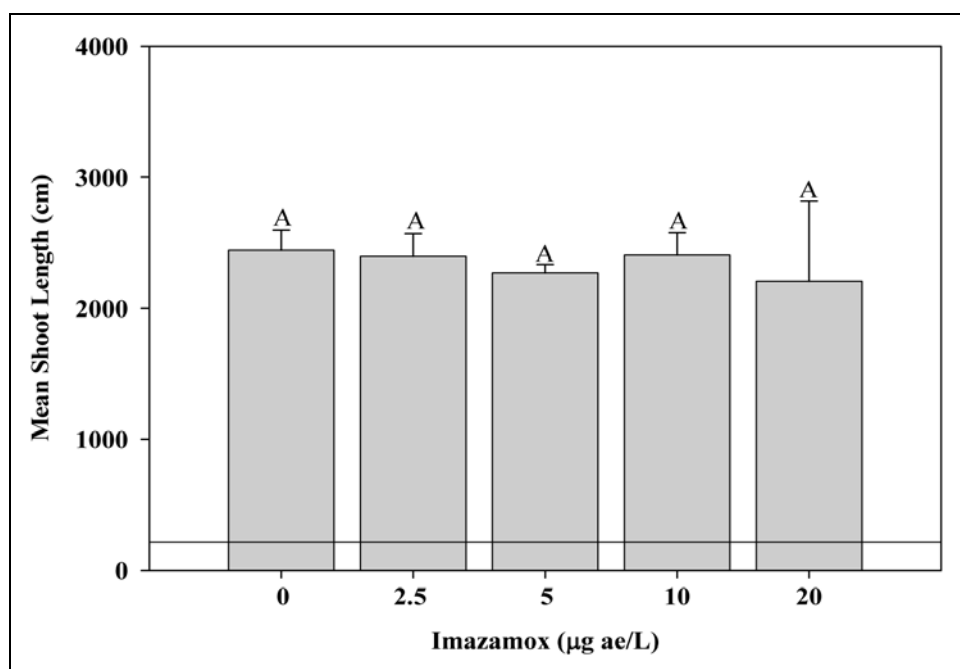


Figure 9. Mean shoot length (cm) of Eurasian watermilfoil treated with imazamox. Shoot length was harvested 45 d after treatment. The horizontal line represents mean pretreatment shoot length and the letters above error bars indicate significant differences between herbicide rates (Holm-Sidak test,  $p \leq 0.05$ ,  $n = 4$ ).

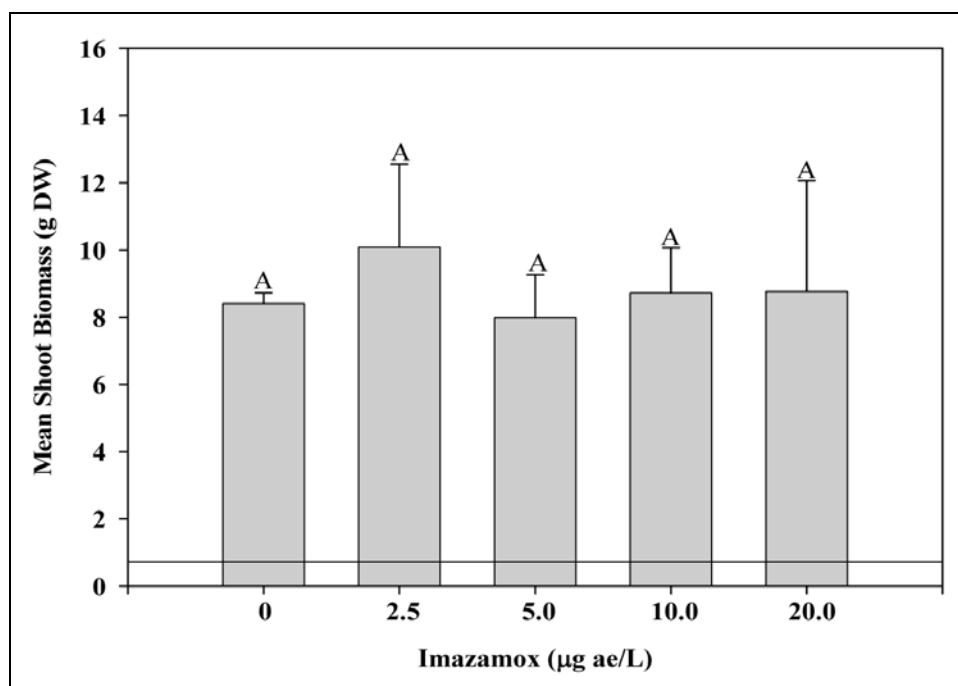


Figure 10. Mean shoot biomass (g DW) of Eurasian watermilfoil treated with imazamox. Biomass was harvested 45 d after treatment. The horizontal line represents mean pretreatment biomass and the letters above the error bars indicate significant differences between herbicide rates (Holm-Sidak test,  $p \leq 0.05$ ,  $n = 4$ ).

## Study 8B

### Materials and Methods

The purpose of this second study was to evaluate two higher rates of imazamox against Eurasian watermilfoil that were not evaluated in Study 8A, and at a longer exposure period. This study was also conducted at the ERDC in a controlled-environment growth chamber (58 m<sup>2</sup>). Experimental conditions within the chamber were maintained to mimic ambient conditions conducive for submersed plant growth: water temperature of  $24 \pm 1$  °C, light intensity of  $300 \pm 80$  µmol/m<sup>2</sup>/sec, and a photoperiod of 14:10-h light:dark cycle.

Eurasian watermilfoil was collected from outdoor ponds located at the LAERF. Three apical tips (15 cm length) were planted in each beaker (300 ml), filled with sediment collected from a nearby lake and amended with ammonium chloride at a rate of 0.2 g/L and Osmocote® (18-6-12) at a rate of 4 g/L. Six planted beakers were placed in each of 12 vertical aquaria (50 L) filled with 48 L of culture solution (Smart and Barko 1985). A 1-cm layer of silica sand was added to the sediment surface to prevent suspension of sediment particles in the water column. Plants were allowed to establish



under these conditions for approximately 3 wk prior to treatment. At time of treatment, plants were green, healthy and actively growing.

A stock solution of imazamox (formulated as Clearcast) was prepared. From this stock, two rates of imazamox (40 and 80 µg ae/L) were applied to aquaria and a static exposure was imposed. Untreated reference aquaria were included to evaluate plant growth in absence of herbicide.

To determine pretreatment biomass levels, one day prior to treatment, shoots were harvested from two beakers per treatment level. Post treatment biomass measurements were made by harvesting two additional beakers from each treatment at 6 and 11 WAT. After each harvest, all shoot material was dried at 70 °C for 48 h to obtain a biomass (g DW) measurement for each replicate.

Imazamox treatments were assigned to aquaria in a completely randomized manner and replicated four times. Means for each replicate were calculated from post-treatment harvest data, and then subjected to a one-way ANOVA using Sigmastat (version 3.1). If the assumptions of normality and equal variance were not met, data was analyzed using the Kruskal-Wallis one-way ANOVA based on ranks. If effects were significant ( $p \leq 0.05$ ), means were separated using the S-N-K method.

## Results and Discussion

Shoot length of Eurasian watermilfoil was significantly less than the untreated reference for both rates of imazamox applied. At 6 WAT, shoot length of Eurasian watermilfoil treated with imazamox at 40 and 80 µg ae/L was 34% and 55% less than the untreated reference, respectively, while at 11 WAT shoot length of Eurasian watermilfoil treated at 40 and 80 µg ae/L was 43% and 71% less than the untreated reference, respectively (Figure 11).

Compared to the untreated reference, shoot biomass was not significantly less at either 6 or 11 WAT (Figure 12). However, there was a slight decrease in shoot biomass at the 11 wk harvest. At that time, Eurasian watermilfoil biomass from the 40 and 80 µg ae/L imazamox treatments were 11% and 25% less than the untreated reference, respectively.

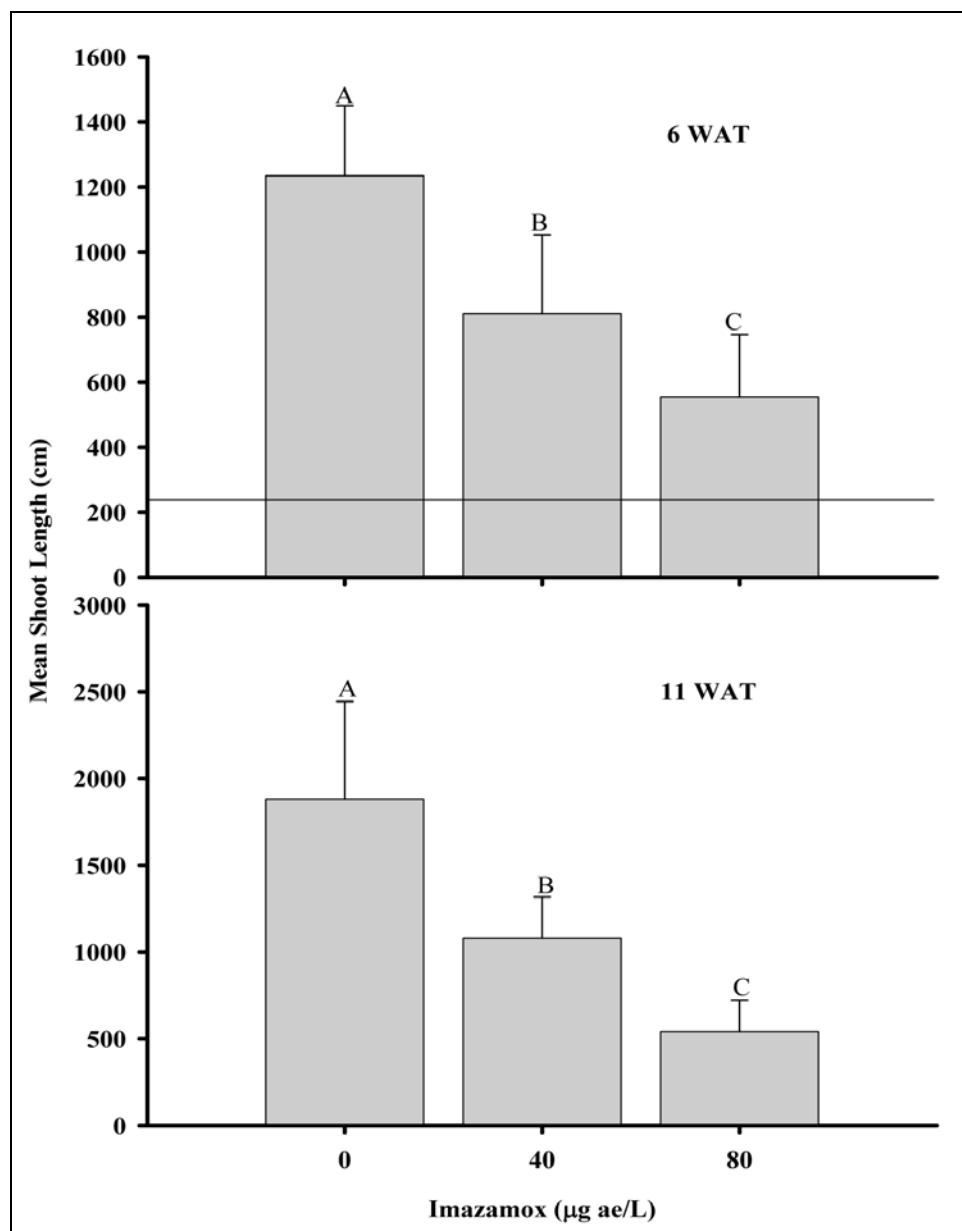


Figure 11 Mean shoot length (cm) of Eurasian watermilfoil 6 and 11 wk after treatment (WAT) to a static exposure of imazamox. Vertical bars represent the mean of four replicates. The horizontal line at 6 WAT represents mean pretreatment shoot length. The letters indicate significant treatment differences according to the Student-Newman-Keuls (S-N-K) method ( $p \leq 0.05$ ).

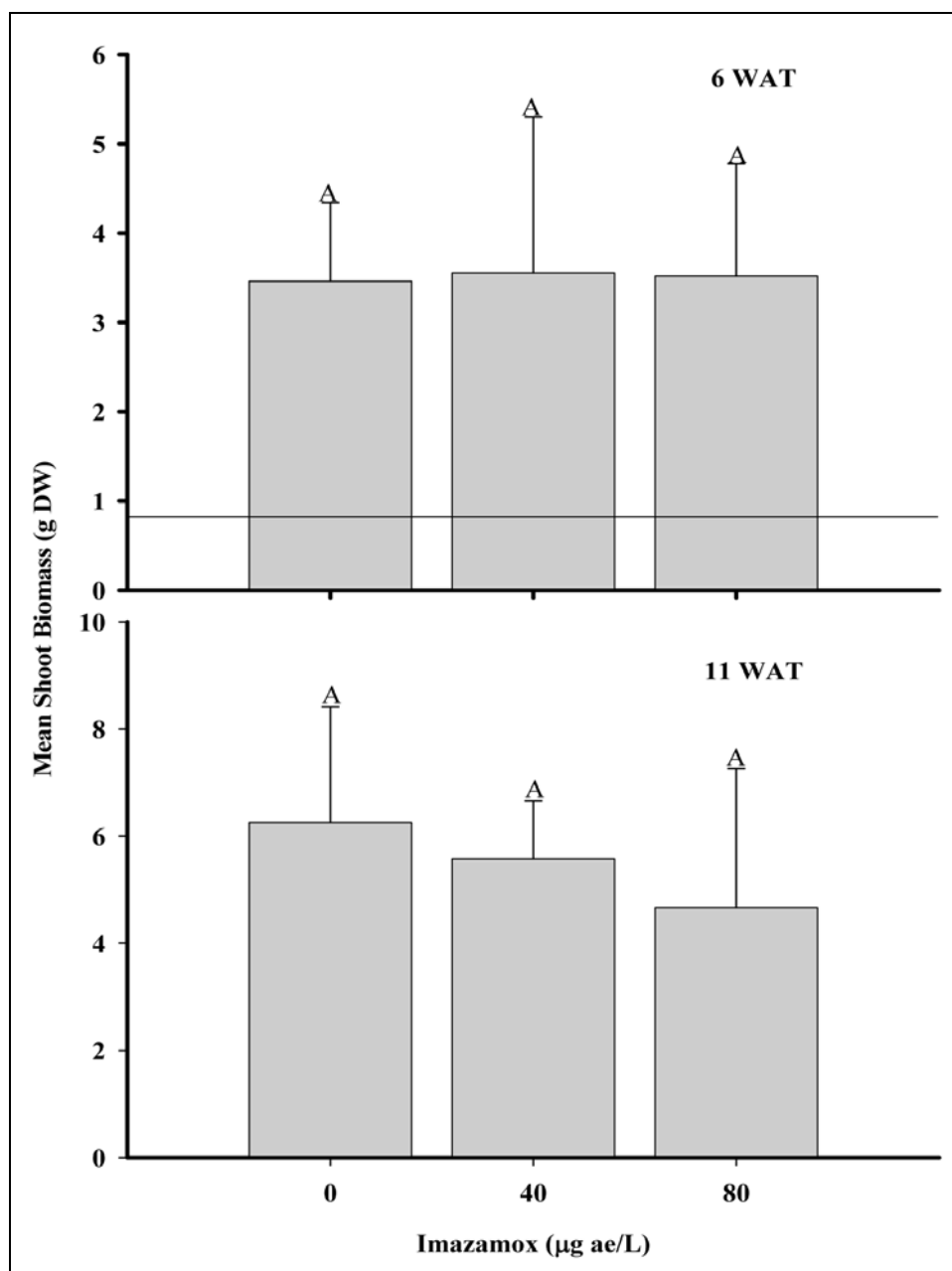


Figure 12. Mean shoot biomass (g DW) of Eurasian watermilfoil 6 and 11 wk after treatment (WAT) to a static exposure of imazamox. The vertical bars represent the mean of four replicates. The horizontal line at 6 WAT represents mean pretreatment shoot biomass. Letters indicate significant treatment differences according to the Student-Newman-Keuls (S-N-K) method ( $p \leq 0.05$ ).

While the higher imazamox rates used in this study resulted in reduction of shoot length, shoot biomass levels were essentially the same (no significant reduction). These results indicate differential response and activity of imazamox against Eurasian watermilfoil; however, additional concentrations and exposure times need to be evaluated to refine

application rates with respect to injury, plant growth regulatory effects and plant death. Higher concentrations with longer exposure times might provide better control and completely kill the plant. The fact that higher herbicide concentrations produced shoots that were shorter, without significant decreases in biomass, suggests the potential of imazamox as a plant growth regulator (PGR). Studies that focus on how imazamox could be used as a PGR should also be conducted.

## Study 8C

### Materials and Methods

Eurasian watermilfoil was collected from Lake Gaston, NC, and planted in 15-cm deep pots containing a sandy loam soil typical of the region. Pots were placed in 73 L tubs containing 70 L of pond water. Each tub contained one 15-cm pot of Eurasian watermilfoil with a shoot average height of 30 cm. An imazamox stock solution (formulated as Clearcast®) calculated by percent ai was prepared. From this stock solution, in-water treatments were applied using a pipette and included imazamox at 0, 25, 50, 75, 100 and 200 ug/L and a sequential application of imazamox at 25 ug/L repeated every 4 wk. Each treatment was replicated four times. Plant control was visually rated on a 0 to 100% scale with 0% equal to no control and 100% equal to complete plant death. Ratings were taken monthly for four months. Plant height was also measured and plants were harvested for dry weight determination. After initial harvest, a second harvest was conducted four weeks later to measure weed regrowth. All data was subjected to analysis of variance, and was means separated according to Fisher's Projected LSD ( $P < 0.05$ ).

### Results and Discussion

At 1 MAT, Eurasian watermilfoil control did not exceed 47% with imazamox (Table 8). At approximately 2 MAT, control was 70 to 72% with 100 and 200 ug/L imazamox, although by 3 MAT, control with 75 to 200 ug/L imazamox was 57 to 65%. Eurasian watermilfoil height was 24 to 30 cm at 1.5 MAT and lower than height of control plants (63 cm). By 3 MAT, little difference in maximum shoot height was present between imazamox treatments and the untreated control. Dry weight biomass was 5.2 to 5.5 g with 75 to 200 ug/L imazamox, and 10.2 g with the untreated, respectively. Regrowth dry weight was variable with no treatment differences. In summary, imazamox at 75 to 200 ug/L suppressed Eurasian watermilfoil

Table 8. Eurasian watermilfoil response to selected imazamox treatments.

Pest Type										
Pest Code										
Pest Name				E. Milfoil	E. Milfoil	E. Milfoil	E. Milfoil	E. Milfoil	E. Milfoil	E. Milfoil
Part Rated										Regrowth
Rating Date				7/21/2006	8/3/2006	8/9/2006	9/12/2006	9/13/2006	9/12/2006	10/12/2006
Rating Data Type				Control	Height	Control	Control	Height	Dry Weight	Dry Weight
Rating Unit				0-100%	in	0-100%	0-100%	inch	g	g
Trt No.	Treatment Name	Rate	Rate Unit							
1	Nontreated Control			0	25.00	0	0	25.25	10.19	0.47
2	Imazamox*Clearcast	25	ppb a	17	11.00	58	37	27.83	16.09	0.27
3	Imazamox*Clearcast	50	ppb a	37	9.67	56	37	25.00	9.53	0.27
4	Imazamox*Clearcast	75	ppb a	28	11.00	55	65	13.43	5.47	0.30
5	Imazamox*Clearcast	100	ppb a	37	9.33	70	57	21.58	5.23	0.20
6	Imazamox*Clearcast	200	ppb a	47	9.33	72	63	17.65	6.34	0.50
7	Imazamox*Clearcast	25	ppb a	15	11.67	30	30	25.42	12.30	0.53
	Imazamox*Clearcast	25	ppb a							
	Imazamox*Clearcast	25	ppb a							
	Imazamox*Clearcast	25	ppb a							
LSD (P=.05)				24.0	3.593	20.4	25.0	13.538	14.680	0.741
Standard Deviation				13.7	2.051	11.5	14.2	7.676	8.324	0.423
CV				44.73	17.46	21.01	29.22	39.32	102.22	133.6

growth, although this would probably not be considered operationally acceptable. Control reached a maximum level at about 2 MAT, but then declined by 3 MAT. Higher rates or multiple applications should be evaluated in order to determine maximized control. In addition, imazamox treatment followed by a contact herbicide application should be evaluated for improving long-term control.

## **Study 9. Eurasian Watermilfoil Treated with Penoxsulam**

**Objective:** To evaluate efficacy of penoxsulam against Eurasian watermilfoil under a long herbicide exposure time regime.

### **Materials and Methods**

This study was conducted at the ERDC in a controlled-environment growth chamber (58 m<sup>2</sup>). Within the chamber, 20 vertical aquaria (50 L) were used to evaluate the herbicide penoxsulam against Eurasian watermilfoil. Conditions within the growth chamber were maintained at  $24 \pm 1$  °C with a light intensity of  $311 \pm 75$   $\mu\text{mol}/\text{m}^2/\text{sec}$  and a 10:14-h light:dark photoperiod.

Eurasian watermilfoil was collected from Medicine Lake, Minnesota. After rinsing all plant material, three apical stems (15 cm length) were planted in each beaker (450 ml) filled with sediment collected from a nearby lake and amended with ammonium chloride at a rate of 0.2 g L<sup>-1</sup>. A thin layer (0.5 cm) of silica sand was then added to the sediment surface to reduce sediment and nutrient dispersion into the water column. Five planted beakers were placed in each of 60 aquaria and filled with 48 L of culture solution (Smart and Barko 1985). To maximize herbicide uptake, plants were treated before canopy formation three weeks after planting when shoots had just reached the water's surface.

Prior to herbicide treatment, one beaker was harvested from each aquarium to obtain a shoot length and biomass pretreatment measurement. Shoot length was measured by cutting all plant material from the surface of the sediment within each beaker and all shoots were measured to the nearest centimeter. All plant material from each beaker was then oven dried at 70°C for 48 h and weighed to the nearest g DW.

A penoxsulam stock solution (formulated as Galleon®) was prepared. From this stock solution, penoxsulam rates of 2.5, 5, 10, and 20  $\mu\text{g ae L}^{-1}$  were

applied to treatment aquaria. An additional treatment, which served as a reference with no herbicide ( $0 \mu\text{g ae L}^{-1}$ ) was also present. At 45 DAT, the four remaining beakers in each aquaria were removed. Total shoot length was recorded and plants were dried at  $70^\circ\text{C}$  for 48 h to obtain biomass measurement. Shoot length and shoot biomass are reported as the sum of four beakers total length (cm) and the sum of four beakers (g DW), respectively.

Each treatment was replicated four times. If shoot length or biomass data did not meet the assumptions of normality or equal variance, data was square root transformed, then reanalyzed using one-way ANOVA to determine differences in herbicide concentrations ( $p \leq 0.05$ ). If significant treatments were detected, the Holm-Sidak means comparison procedure was performed.

## Results and Discussion

All rates of penoxsulam significantly reduced shoot length of Eurasian watermilfoil compared to the untreated reference (Figure 13), with shoot length reduction ranging from 16 to 85%. Additionally, all rates of penoxsulam, except the  $2.5 \mu\text{g ae L}^{-1}$  rate, significantly reduced shoot biomass compared to the untreated reference (Figure 14), with biomass reduction ranging from 14 to 87%. There was a 7% increase in shoot biomass in the  $2.5 \mu\text{g ae L}^{-1}$  treatment. Results indicate that penoxsulam applied at rates  $> 10 \mu\text{g ae L}^{-1}$  and for exposure periods of 45 d will be necessary to adequately control Eurasian watermilfoil. Additional refinement of CET relationships, particularly with respect to non-target plant impacts, will be required before recommendations can be made for wide-spread use of this product on Lake Gaston.

## Egeria – Nuisance Exotic Species

Summarized results from previous studies using diquat, are provided below:

**Diquat.** The contact herbicide diquat (as Reward®) is effective in controlling egeria (aka Brazilian elodea) with  $>90\%$  shoot biomass reductions using labeled rates under exposure times as short as 5 h (Skogerboe et al. 2006). The application window for optimum plant control is in late spring when plants are actively growing and water temperature is above  $12^\circ\text{C}$ . Suppression of egeria has been maintained for 6 wk in a

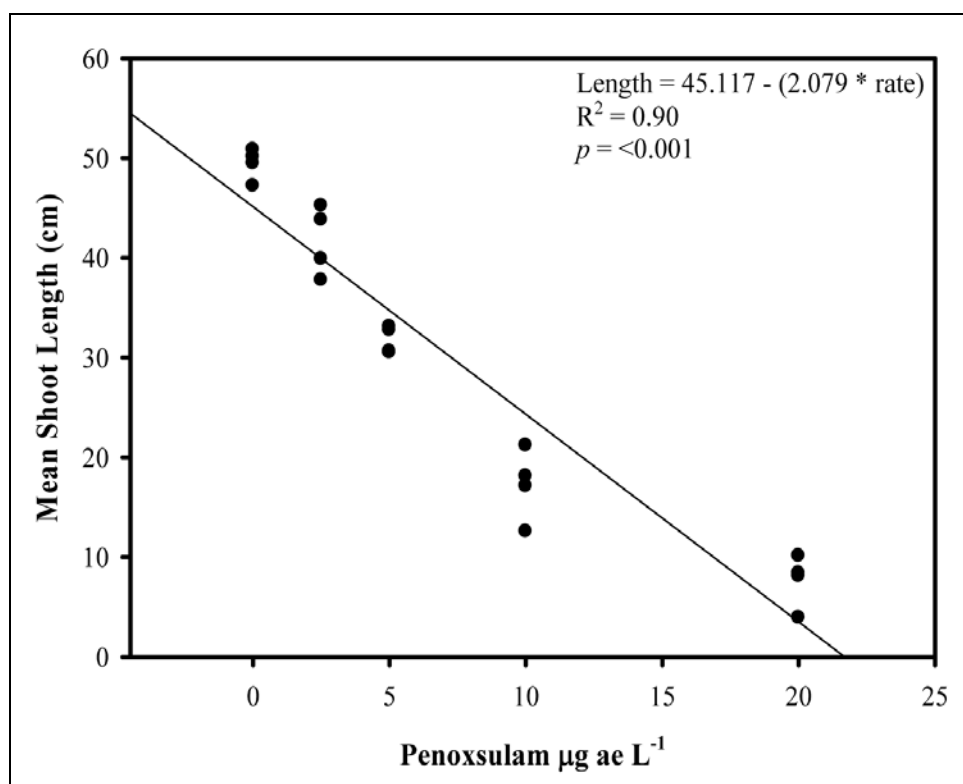


Figure 13. Regression of mean shoot length (cm) to penoxsulam ( $\mu\text{g ae L}^{-1}$ ) for Eurasian watermilfoil 45 d after treatment.

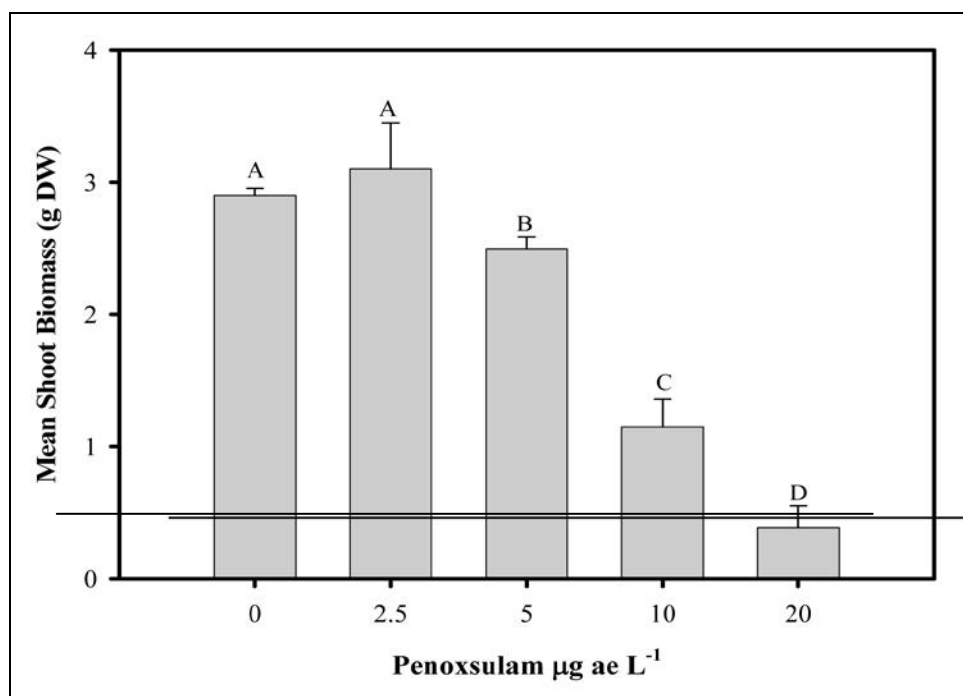


Figure 14. Mean shoot biomass (g DW) of Eurasian watermilfoil treated with penoxsulam. Biomass was harvested 45 d after treatment. The horizontal line represents mean pretreatment biomass and the letters above the error bars indicate significant differences between herbicide rates (Holm-Sidak test,  $p \leq 0.05$ ,  $n = 4$ ).



small-scale study without regrowth (Poovey and Getsinger 2002). Because diquat is a contact herbicide, only killing the leaves and stems, regrowth would be expected after one growing season. In addition, since diquat is readily and tightly bound to mineral clays and organic matter, this herbicide is most effective when used in clear water. Use of diquat in turbid water conditions will inactivate the product and result in poor or no control of treated vegetation (Hofstra et al. 2001; Poovey and Getsinger 2002). The most appropriate use of diquat for egeria control in Lake Gaston would be for relatively small-scale, partial lake applications, when plants are young and actively growing in non-turbid water conditions where broad spectrum removal of submersed aquatic plants in those settings would only represent a small proportion of the total lake plant community.

### **Other Submersed Macrophytes**

#### *Vallisneria – Native Species*

*Vallisneria* (wild celery, eel grass) is a native plant and is considered a valuable component of the submersed plant community of Lake Gaston. Therefore, it would be considered a non-target plant species when treating invasive plant populations on the lake. Herbicide CETs summarized in this section are for the contact herbicide endothall and the systemic herbicides, triclopyr and fluridone.

#### *Endothall*

Results from previous studies to determine the species' selective properties of endothall (as Aquathol® K) indicated that *vallisneria* is somewhat sensitive to the herbicide, but recovery from an application of endothall is likely (Skogerboe and Getsinger 2001, 2002). In these studies, endothall rates of 0.5, 1, 2, 4, and 5 mg/L with an exposure time consisting of a 24-h flow through half-life for 7 d were evaluated. A significant reduction of *vallisneria* shoot biomass was measured ranging from 60 to 75 % by 6 to 8 WAT. However, at the conclusion of the studies, green, healthy, *vallisneria* shoot tissue was present indicating a strong likelihood that the plant would recover and regrow following an exposure to endothall. Based on the results of these studies, caution should be used when applying endothall where *vallisneria* is a species of concern.

*Triclopyr*

Results from previously conducted outdoor mesocosm evaluations showed that triclopyr (as Renovate®) at application rates of 0.5 to 2.5 mg ae/L caused little injury to vallisneria (Smart et al. 1995). Field studies on Lake Minnetonka, Minnesota, verified results of the mesocosm evaluations, showing that triclopyr applications of 0.5 to 2.5 mg ae/L did not significantly impact vallisneria (Petty et al. 1998, Poovey et al. 2004). Since triclopyr is selective for dicots, exposure to even the highest allowable rate of triclopyr (2.5 mg/L) should cause little or no injury to a monocot such as vallisneria.

*Fluridone*

Several previously published small-scale studies evaluating the species-selective potential of fluridone (as Sonar® AS or AVAST!®, SePRO Corp., Carmel, IN) were conducted against vallisneria (Netherland et al. 1997, Nelson et al. 1998, Poovey et al. 2004, Sprecher et al. 1998). In a growth chamber study (Sprecher et al. 1998), fluridone applied at rates of 2, 10 and 25 µg ai/L with a static exposure of 90 d were evaluated. At 90 DAT, a significant reduction in shoot biomass of vallisneria was measured in 10 and 25 µg ai/L, reducing biomass by 85% and 89%, respectively. There was also a decrease in shoot biomass in the lowest fluridone treatment (2 µg ai/L); however, it was only reduced by 3% compared to the untreated reference, and was not significant.

In an outdoor mesocosm study (Netherland et al. 1997), fluridone rates of 5, 10 and 20 µg ai/L with a half-life of 33 d (60 and 90d) were evaluated. Vallisneria shoot biomass was significantly reduced in treatments  $\geq 10$  µg ai/L at both the 60 and 90 DAT harvests. Shoot mass reported in the 5 µg ai/L fluridone rate was also significantly reduced. However, at this low rate, plants at both exposure periods had a greater biomass than the untreated reference, suggesting that recovery and regrowth of vallisneria would be likely. In a second outdoor mesocosm study (Nelson et al. 1998), fluridone applied at a rate of 5 µg ai/L with a half-life of 49 d (21-, 42- and 84-d exposure) was evaluated. Results from this evaluation showed that vallisneria shoot mass was not controlled at any of the three exposure periods. In a third outdoor mesocosm study (Poovey et al. 2004), fluridone applied at rates of 6, 12 and 24 µg ai/L with a half-life of 23 d (56-d exposure) were evaluated. At 56 DAT, no significant decrease in shoot biomass of vallisneria was measured at any of the three rates evaluated.

The two lower rates (6 and 12 µg ai/L) actually had an increase in shoot biomass compared to the untreated reference, while the 24 µg ai/L had a negligible reduction.

While fluridone evaluations reported from growth chamber and outdoor mesocosm studies indicate that herbicide injury should be minimal on vallisneria, at rates  $\leq 12$  µg ai/L, some discrepancy has been observed in field verification studies. Vallisneria occurrence increased substantially in whole-lake fluridone applications of 5 µg ai/L in Michigan (Getsinger et al. 2001); however, a decrease in occurrence of vallisneria was reported from whole-lake applications (6 µg ai/L) in Vermont (Getsinger et al. 2002b). It was not clear if this decrease was caused by a phytotoxic response to fluridone, or from indirect effects of lower light levels measured in the water column following herbicide treatment that inhibited vallisneria growth. Based upon the results of these small- and field-scale evaluations, caution should be used when applying fluridone at rates  $\geq 5$  µg ai/L, if vallisneria is a species of concern in the treatment area.

### **Current Evaluations to Determine CET Information**

Herbicide CETs evaluated in this section (Studies 10 and 11) include the systemic herbicides, bispyribac-sodium and imazamox.

## **Study 10. Vallisneria Treated with Bispyribac-sodium**

**Objective:** To determine if use rates of bispyribac-sodium will injure the native species, vallisneria, under extended exposure times.

### **Materials and Methods**

This study was conducted at the RDC in a controlled-environment growth chamber (58 m<sup>2</sup>). Experimental conditions within the chamber were maintained to mimic ambient conditions conducive for submersed plant growth: water temperature of  $24 \pm 1$  °C, light intensity of  $490 \pm 77$  µmol/m<sup>2</sup>/sec, and a photoperiod of 14:10-h light:dark cycle.

Vallisneria was obtained from Suwannee Laboratories, Lake City, Florida. It was sub-sampled, weighed, and the stems were cut to approximately 10 cm in length. At the time of planting, mean weight of plants was  $5.2 \pm 1.2$  g. One vallisneria plant was placed in each beaker (300 ml) filled with sediment collected from a nearby lake, and amended with ammonium chloride at a

rate of 0.2 g/L. After planting, a 1-cm layer of silica sand was added to the sediment surface to prevent sediment and nutrient dispersion into the water column. Three planted beakers were placed in each of the 25 vertical aquaria (10 L) filled with 8 L of culture solution (Smart and Barko 1985). Plants were allowed to establish under these conditions for approximately 7 wk prior to treatment. At the time of treatment, plants were healthy and actively growing.

A stock solution of bispyribac-sodium (formulated as Velocity®), calculated by percent ai was prepared. From this stock, four rates of bispyribac-sodium (10, 20, 40, and 80 µg ai/L) were applied to the aquaria and a static exposure regime was imposed. Untreated references (0 µg ai/L) were included to evaluate plant growth in absence of herbicide.

One day prior to herbicide application, a harvest of all shoots from one beaker from each aquarium was performed. Posttreatment biomass measurements were made by harvesting one additional beaker from each aquarium at 6 and 10 WAT. After each harvest, all shoot material was dried at 70 °C for 48 h to obtain a g DW measurement for each replicate. After the 6 WAT harvest, Osmocote® (18-6-12) was added to the remaining beaker at a rate of 2 g/L to mitigate nutrient limitation for the prolonged study period.

Bispyribac-sodium treatments were assigned to aquaria in a completely randomized manner and replicated five times. The means for each replicate were calculated from posttreatment harvest data, and then subjected to a one-way ANOVA using Sigmastat (version 3.1). If the effects were significant ( $p \leq 0.05$ ), the means were separated using the Holm-Sidak means comparison method.

## Results and Discussion

During the final harvest observations, all plants treated with bispyribac-sodium at rates  $\leq 20$  µg ai/L had green leaves and new growth emerging from the root crown. Treatments  $\geq 40$  µg ai/L were reddish-yellow in color, had slight deterioration of the leaves, and no new growth was noted. At 6 WAT, only vallisneria treated at 80 µg ai/L had significantly less biomass than the untreated reference, and by termination of the study there was no difference between the untreated reference and any of the bispyribac-sodium treatments (Figure 15). Additionally, all treatments, including the untreated reference, exhibited a decrease in shoot biomass between the 6 and 10 wk posttreatment harvest.

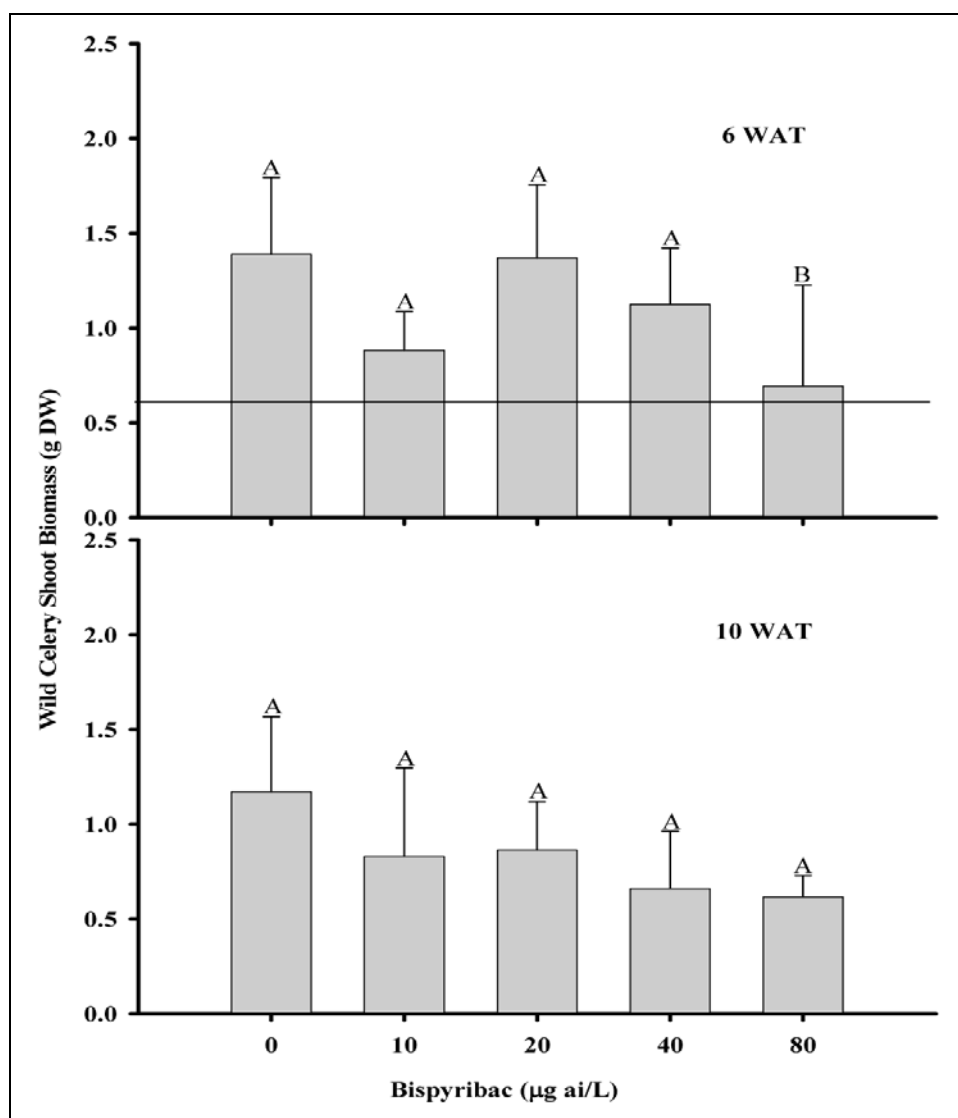


Figure 15. Mean shoot biomass (g DW) of vallisneria celery 6 and 10 wk after treatment (WAT) to a static exposure of bispyribac-sodium. The vertical bars represent the mean of five replicates. The horizontal line at 6 WAT represents mean pre-treatment shoot biomass. The letters indicate significant treatment differences according to the Holm-Sidak method ( $p \leq 0.05$ ).

Based on the results of this study, rates  $\geq 80 \mu\text{g ai/L}$  bispyribac-sodium may be necessary to significantly reduce the shoot biomass of vallisneria. Future evaluations of this herbicide should include these higher rates, as well as contact times exceeding 70 d, to refine bispyribac-sodium CET relationships against vallisneria.

### Study 11. Vallisneria Treated with Imazamox

**Objective:** To determine if use rates of imazamox will injure the native species, vallisneria, under extended exposure times.

## Materials and Methods

This study was conducted at the ERDC in a controlled-environment growth chamber (58 m<sup>2</sup>). Experimental conditions within the chamber were identical to those used in Study 10, above. *Vallisneria* was obtained from Suwannee Laboratories, Lake City, Florida. It was sub-sampled, weighed, and stems were cut to approximately 10 cm in length. At the time of planting, the mean weight of plants was  $5.2 \pm 1.2$  g. One *vallisneria* plant was placed in each beaker (300 ml) filled with sediment collected from a nearby lake and amended with ammonium chloride at a rate of 0.2 g/L. After planting, a 1-cm layer of silica sand was added to the sediment surface to prevent sediment and nutrient dispersion into the water column. Three planted beakers were placed in each of the 25 vertical aquaria (10 L) that were filled with 8 L of culture solution (Smart and Barko 1985). Plants were allowed to establish under these conditions for approximately 7 wk prior to treatment. At the time of treatment, the plants were healthy and actively growing.

A stock solution of imazamox (formulated as Clearcast®) calculated by percent ai was prepared. From this stock, four rates of imazamox (10, 20, 40, and 80 µg ai/L) were applied to aquaria and a static exposure regime was imposed. Untreated references (0 µg ai/L) were included to evaluate plant growth in the absence of herbicide.

One day prior to the herbicide application, all the shoots from one beaker in each aquarium was harvested. Posttreatment biomass measurements were made by harvesting one additional beaker from each aquarium at the 6 and 10 WAT. After each harvest, all shoot material was dried at 70 C for 48 hr to obtain a g DW measurement for each replicate. After the 6 WAT harvest, Osmocote® (18-6-12) was added to the remaining beaker at a rate of 2 g/L to mitigate nutrient limitation for the prolonged study period.

Imazamox treatments were assigned to aquaria in a completely randomized manner and replicated five times. The means for each replicate were calculated from posttreatment harvest data, and then subjected to a one-way ANOVA using Sigmastat (version 3.1). If effects were significant ( $p \leq 0.05$ ), the means were separated using the Holm-Sidak means comparison method.

## Results and Discussion

Plants treated with imazamox at rates  $\leq 40 \mu\text{g ai/L}$  were healthy with green growth (and some slight yellowing), and had some leaves of emerging new growth throughout the study period. However, the imazamox treatment of  $80 \mu\text{g ai/L}$  showed yellowish leaves with some deterioration. There were no statistically significant differences in vallisneria shoot biomass among any treatments, including the untreated reference during the study period (Figure 16). Only a small decrease in shoot mass in the imazamox treatment of  $80 \mu\text{g ai/L}$  at 10 WAT was noted. Based on results of this study, rates  $\geq 80 \mu\text{g ai/L}$  of imazamox may be necessary to significantly reduce shoot biomass of vallisneria. Future evaluations of this herbicide should include these higher rates, as well as contact times exceeding 70 d, to refine imazamox CET relationships against vallisneria.

**Southern Naiad – Native Species.** Southern naiad is a native plant and is considered a valuable component of the submersed plant community of Lake Gaston. Therefore, it would be considered a non-target plant species when treating invasive plant populations on the lake. The herbicide CET relationship summarized in this section (Study 12) is for the systemic herbicide penoxsulam.

### Study 12. Southern Naiad Treated with Penoxsulam

**Objective:** To determine if low-use rate applications of penoxsulam will injure the native species, southern naiad, under an extended exposure time.

#### Materials and Methods

This study was conducted in outdoor mesocosms at NCSU. Southern naiad shoots were collected from field sites in North Carolina. Apical shoots were planted in hydrosol amended with nutrients in 45 x 18 cm flats and placed in 950-L concrete vaults modified for aquatic plant experimentation.

For herbicide applications, a stock solution of penoxsulam (formulated as Galleon®) was prepared. Using this stock solution, penoxsulam treatments included a  $0 \mu\text{g/L}$  (untreated reference); 5, 10 and  $20 \mu\text{g/L}$  with a 90 DET;  $5 \mu\text{g/L}$  with a static exposure period; and  $20 \mu\text{g/L}$  with 45 DET (Table 9). The concentration of treatment 5 was maintained by flushing the tank at 30 and 60 DAT and retreating. Each treatment was replicated 3 times and initial treatments were applied on 28 June 2006.

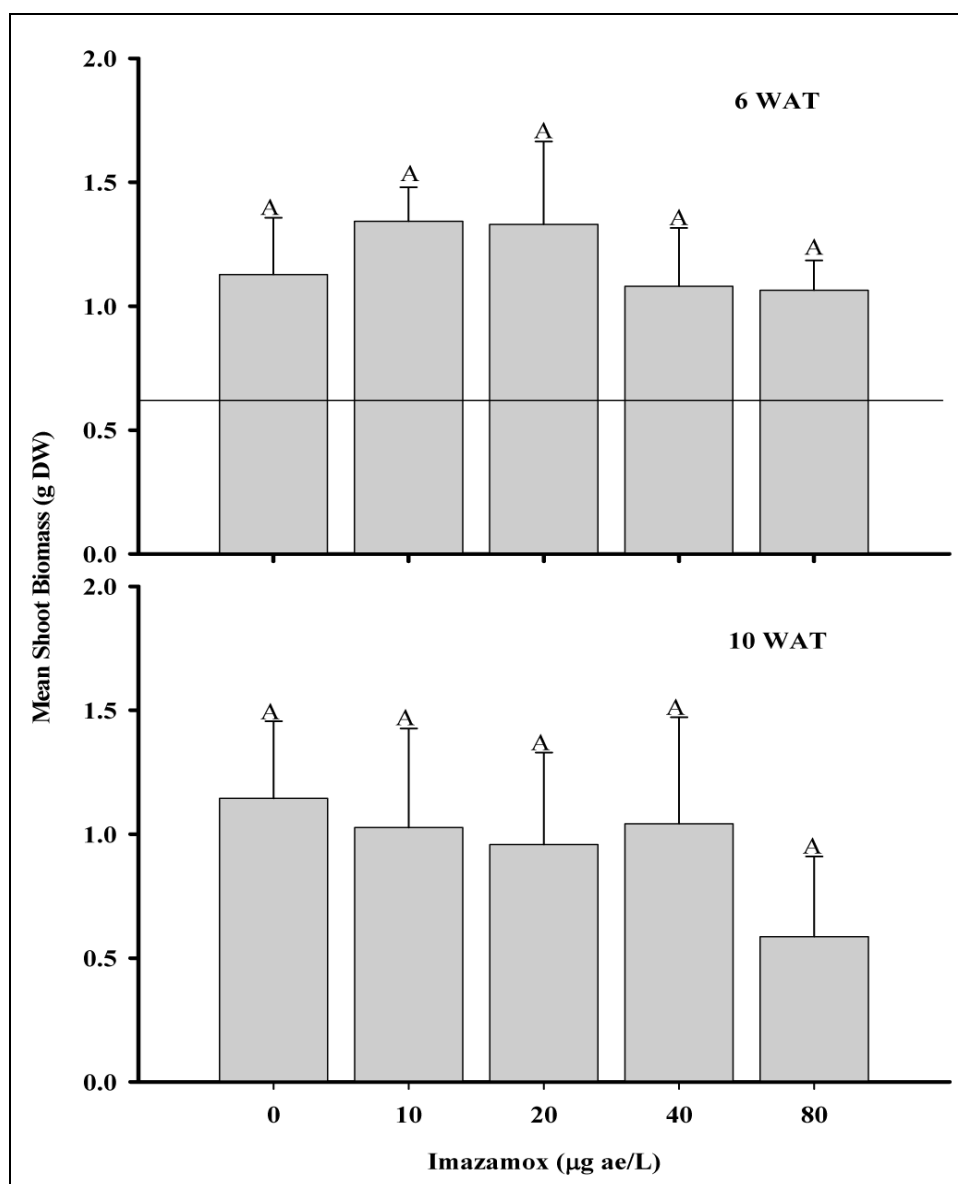


Figure 16. The mean shoot biomass (g DW) of *Vallisneria* 6 and 10 wk after treatment (WAT) to a static exposure of imazamox. The vertical bars represent the mean of five replicates. The horizontal line at 6 WAT represents the mean pretreatment shoot biomass. The letters indicate significant treatment differences according to the Holm-Sidak method ( $p \leq 0.05$ ).

Plant control was visually rated weekly on a 0 to 100% scale with 0% equal to no control and 100% equal to complete plant death. Plant height was measured at approximately 42 and 90 DAT. Plant shoots were harvested at 90 DAT for biomass (g DW) determination. After harvest, flats were placed back into vaults with fresh water and allowed to regrow. Regrowth was harvested at 42 d after initial harvest for biomass determination. All data was subjected to analysis of variance and Fisher's Protected LSD was used for mean separation.



Table 9. Southern naiad response to selected penoxsulam treatments.

Pest Name				S. naiad	S. naiad	S. naiad	S. naiad	S. naiad	S. naiad	S. naiad	S. naiad	S. naiad
Description												
Rating Date				7/5/2006	7/11/2006	7/19/2006	7/26/2006	8/4/2006	8/4/2006	8/8/2006	8/9/2006	8/25/2006
Rating Data Type				Control	Control	Control	Control	Control	Height	Control	Control	Control
Rating Unit				0-100%	0-100%	0-100%	0-100%	0-100%	cm	0-100%	0-100%	0-100%
Trt No.	Treatment Name	Rate	Rate Unit									
1	Nontreated Control			0	0	0	0	0	18	0	0	0
2	Penoxsulam*Galleon	20	ppb a	0	40	52	70	91	4	96	95	100
3	Penoxsulam*Galleon	10	ppb a	0	3	3	8	62	10	37	76	90
4	Penoxsulam*Galleon	5	ppb a	0	5	8	18	63	9	64	79	79
5	Penoxsulam*Galleon	20	ppb a	0	2	17	73	93	3	100	99	100
6	Penoxsulam*Galleon	5	ppb a	0	3	2	10	13	13	0	40	55
7	Penoxsulam*Galleon	10	ppb a	0	3	32	53	65	9	94	94	100
LSD (P=.05)				1.8	18.6	36.8	36.7	48.0	9.4	55.3	33.6	35.9
Standard Deviation				1.0	10.5	20.7	20.6	27.4	5.7	31.6	19.2	20.5
CV				489.9	113.69	128.8	67.83	46.83	62.72	54.57	26.92	26.54
Pest Name				S. naiad	S. naiad	S. naiad	S. naiad					
Description							Regrowth					
Rating Date				9/15/2006	9/15/2006	9/15/2006	11/2/2006					
Rating Data Type				Control	Dry Weight	Height	Dry Weight					
Rating Unit				0-100%	g	inch	g					
Trt No.	Treatment Name	Rate	Rate Unit									
1	Nontreated Control			0	13.4	21.8	1.0					
2	Penoxsulam*Galleon	20	ppb a	100	0.0	0.0	0.0					

Trt No.	Treatment Name	Rate	Rate Unit									
3	Penoxsulam*Galleon	10	ppb a	96	0.2	4.0	1.4					
4	Penoxsulam*Galleon	5	ppb a	71	6.9	14.3	0.0					
5	Penoxsulam*Galleon	20	ppb a	100	0.0	2.7	0.0					
6	Penoxsulam*Galleon	5	ppb a	57	12.4	18.2	0.0					
7	Penoxsulam*Galleon	10	ppb a	100	0.0	0.0	0.0					
LSD (P=.05)				36.6	9.96	12.56	1.72					
Standard Deviation				23.7	7.50	8.31	0.98					
CV				31.89	143.4	102.3	326.6					

## Results and Discussion

At 35 DAT, southern naiad control was 91 to 93% with 20 ug/L penoxsulam at both exposure times. (Table 9). Other penoxsulam treatments controlled this species 13 to 65%. Naiad height was 3 to 4 cm with 20 ug/L penoxsulam, but was 9 to 13 cm with other penoxsulam treatments. At 90 DAT, southern naiad was controlled 88 to 100% with all penoxsulam rates greater than 5 ug/L. Sensitivity of this species to penoxsulam was also reflected by low dry weights with treatments. Only dry weights from the 5 ug/L penoxsulam treatments were similar to the untreated reference (i.e., a control). Dry weights with other treatments did not exceed 0.4 g. Height of naiad also reflected control, with all penoxsulam rates greater than 5 ug/L limiting height to  $\leq 10$  cm. Southern naiad did not survive initial harvest well, thus no differences in regrowth weight were present.

Southern naiad seems to be sensitive to rates of penoxsulam that might be used to control invasive plants, such as hydrilla or Eurasian watermilfoil. Thus, other treatment options might be more desirable to remove hydrilla from stands of southern naiad when that species is of concern in areas of Lake Gaston. Additional work should be conducted to confirm southern naiad's response to penoxsulam.

## Lyngbya – Nuisance Alga

### Current Evaluations - Study A. Chelated Copper and Endothall

**Objective:** To evaluate the efficacy of two formulations of chelated copper and one formulation of endothall, alone and in combination, on the blue green alga, lyngbya.

#### *Materials and Methods*

This study was conducted at the LAERF in a benchtop environmental growth chamber. Conditions in the chamber were maintained to provide healthy lyngbya growth as follows: 90  $\mu\text{mol photons/m}^2/\text{s}$ , 25 °C and 14:10h light:dark photoperiod.

Lyngbya was collected from Lake Cypress Springs, Texas. Small, moderately dense mats of lyngbya (~ 2 cm in diameter) were placed into 250 mL flasks containing 150 mL of CLII growth medium and treated with selected algaecides. Algaecide applications included single applications at 1.0 mg/L copper (from a chelated copper stock solution formulated as K-Tea®),

SePRO Corp., Carmel, IN); 1.0 mg/L copper (from a chelated copper stock solution formulated as Clearigate®, Applied Biochemists, Germantown, WI); and 1.5 and 3 mg/L endothall (from an amine stock solution formulated as Hydrothol®, United Phosphorus International, King of Prussia, PA). Algaecide combination treatments included 1.0 mg/L copper (K-Tea®) + 1.5 mg/L endothall; 1.0 mg/L copper (K-Tea®) + 3.0 mg/L endothall, 1.0 mg/L copper (Clearigate®) + 0.5 ppm endothall, 1.0 mg/L copper (Clearigate®) + 1.0 mg/L endothall, and 1.0 mg/L copper (Clearigate®) + 3.0 mg/L endothall. The treatments were replicated three times and included an untreated reference (i.e., a control). At 14 DAT, lyngbya was harvested from each flask, dried at 65 °C and weighed.

### *Results and Discussion*

At 4 DAT, only lyngbya in the untreated control flasks was dark green and floating on the surface of the culture medium. In all other treatments, the lyngbya mats were pale green with some browning and had fallen to the bottom of the flask. By 10 DAT, however, the lyngbya treated at 1.5 mg/L endothall and one flask treated at 3.0 mg/L endothall were recovering (mats turning green and floating on surface again).

Lyngbya mats treated with copper + endothall combinations were brown and starting to bleach in spots; however, there was still viable tissue remaining at 14 DAT, and biomass was only reduced by 41 and 60 %, respectively (Figure 17). The copper applications of 1.0 mg/L (K-tea® or Clearigate®) alone resulted in a 60 and 69 % decrease in biomass. Percent control for all combinations of chelated copper + endothall was 61 to 66 %.

The results from this study do not indicate increased efficacy against lyngbya when combining chelated copper products with endothall. The density of lyngbya filaments found in mats may inhibit the penetration of herbicides such as copper and endothall throughout the entire mat, thus reducing efficacy. Instead, only the outer layer of algae is affected by the herbicide application, which may lead to recovery and regrowth of lyngbya under field conditions.

## **Current Evaluations - Study B. Chelated Copper**

**Objective:** To evaluate the efficacy of a chelated copper formulation on the bluegreen alga, lyngbya.

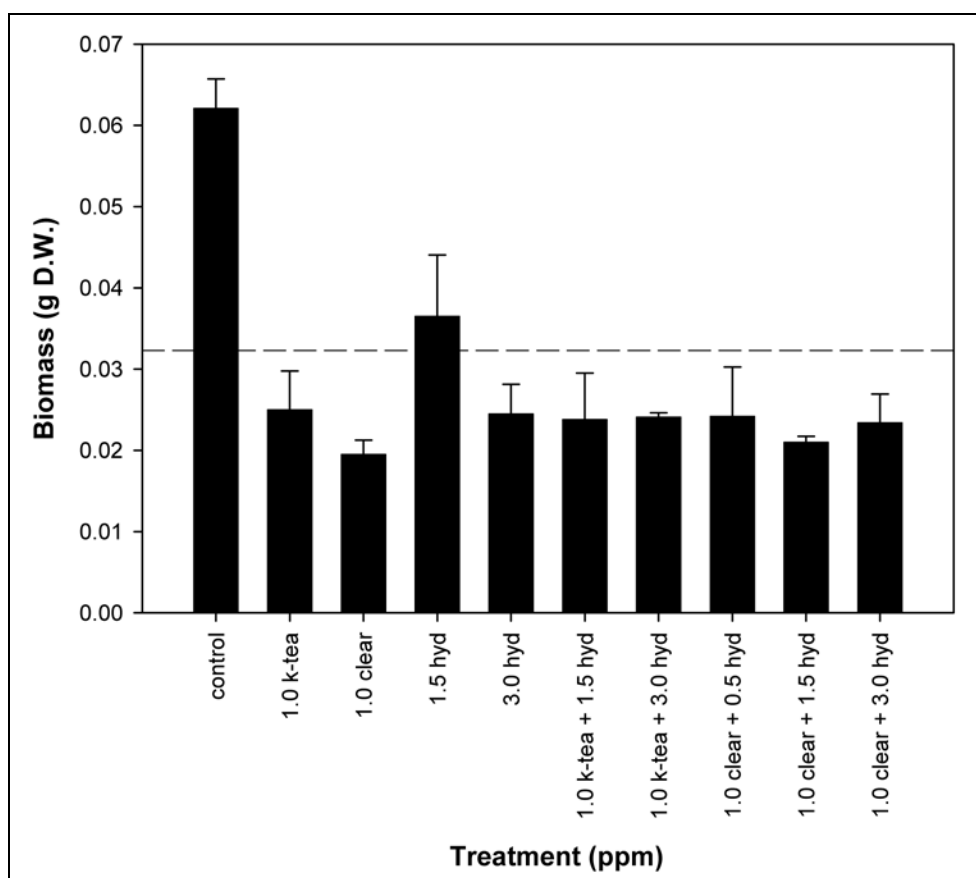


Figure 17. Lyngbya biomass ( $\pm$  SE) at 14 days after treatment. The dashed line represents initial, pretreatment biomass. Treatments include 1.0 mg/L copper (K-Tea®), 1.0 mg/L copper (Clearigate®), 1.5 and 3.0 mg/L endothall (Hydrothol®) and combinations of copper + endothall.

## Materials and Methods

This study was conducted in a greenhouse at the LAERF. Lyngbya was collected from Lake Cypress Springs, Texas. Small, moderately dense mats of lyngbya about 1 cm<sup>2</sup> in diameter were placed into 250 mL specimen cups containing 240 mL of CLII medium. The specimen cups were set inside a water bath and temperatures were maintained at 25 °C. The lyngbya mats were teased apart to improve exposure of lyngbya filaments to the algacide treatments. A copper stock solution (formulated as Clearigate®) was prepared. From this stock solution, rates of 0.1, 0.25, 0.5, 0.75, 1.0, 1.25, and 1.5 mg/L copper were used at treatments. Treatments were replicated three times and included an untreated control. At 7 DAT lyngbya was harvested from each flask by pouring the medium over a preweighed glass fiber filter (47 mm), dried at 65 °C, and weighed.

## Results and Discussion

At the time of harvest, green filaments were still present in the cups treated at 0.1 and 0.25 mg/L copper, and percent control for these treatments was 15 and 35 %, respectively (Figure 18). At 0.5 mg/L, there was partial bleaching of the lyngbya filaments and control was 69 %. At rates higher than 0.5 mg/L, many of the lyngbya filaments were bleached and control ranged from 74 to 78 %. Copper at 0.75 mg/L provided moderate control of lyngbya (74%), and no improvement in efficacy was measured at rates > 0.75 mg/L.

Results from this study show that lyngbya is susceptible to some forms of chelated copper, and slightly greater control (up to 79 %) was provided against mats where filaments were teased apart, versus the control reported in Study A (up to 69 %) in more dense mats. However, due to the density of lyngbya mats in field situations, and the possible limitation of copper to completely penetrate the mat, repeat applications of the chelated copper may be required to provide adequate control of this nuisance alga in Lake Gaston.

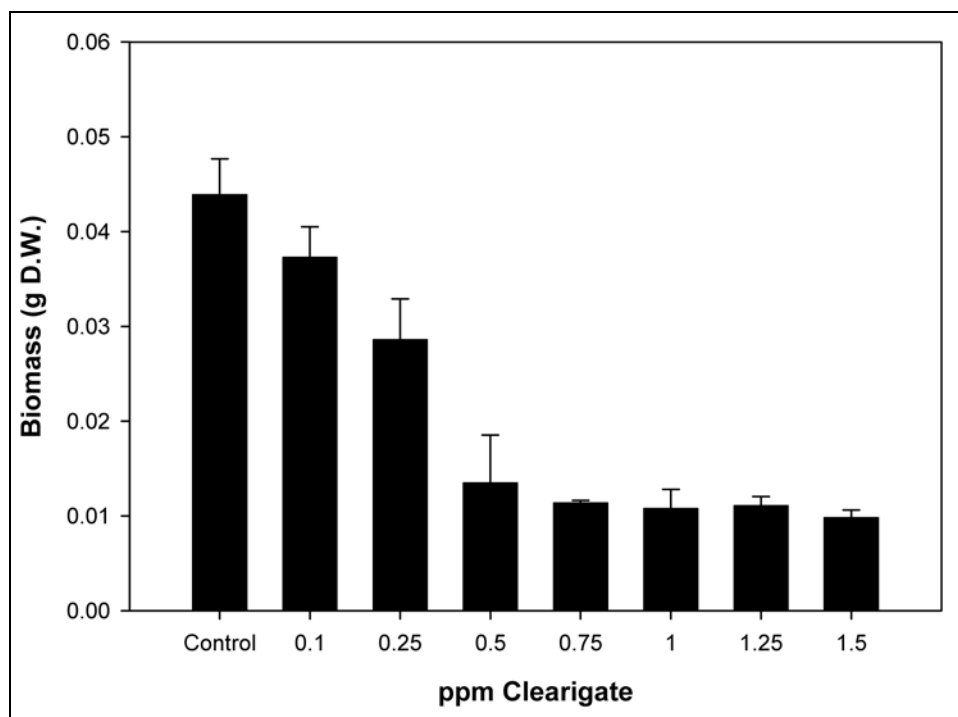


Figure 18. Lyngbya biomass ( $\pm$  SE) at 7 d after treatment with chelated copper (Clearigate®).

## Interim guidance for chemical control on Lake Gaston

The concept of aqueous herbicide CET relationships for selectively controlling submersed invasive plants is integral to the operational success of prescriptive herbicide treatments. This CET information is best used when coupled with water exchange processes in specific application sites. When linked in this way, prescriptive treatments can be used to control harmful invasive plants while minimizing damage to beneficial native vegetation. This management strategy is known as species-selective control.

Species-selective control strategies are dependent upon scientific studies that define and document CET relationships for each herbicide against specific invasive plants and non-target plants. To develop prescriptive herbicide treatments for successful and consistent species-selective control on Lake Gaston, several important factors must be used in concert with them:

1. detailed knowledge of the location of invasive plant populations on the lake, including species occurrence and abundance;
2. adequate understanding of CET relationships for each aquatic herbicide against the invasive plants and impacts on key non-target plants;
3. coupling of CET information with knowledge of water exchange patterns in plant stands targeted for chemical applications, and;
4. quantitative pre- and posttreatment assessment of the plant communities to determine herbicide effectiveness.

Based on the CET information in this report -- which is only one of several key factors required for developing prescriptive treatments -- interim guidance is provided for the selective chemical control of monoecious and dioecious hydrilla, Eurasian watermilfoil, egeria, and lyngbya on Lake Gaston. Recommendations for specific herbicides should only be viewed as a “best fit” based on current information. It should also be noted that use restrictions for each specific herbicide may limit the application of such products on the lake. Moreover, this chemical control guidance can be refined once water exchange processes are defined for specific application sites on Lake Gaston.

## **Monoecious and Dioecious Hydrilla**

### *Diquat*

This product is useful in areas where herbicide contact times are greatly limited (e.g., 3 to 12 h) due to water exchange processes. When used alone, diquat may only provide 80% control of monoecious hydrilla in the year of treatment; therefore, tank mixing of chelated copper may improve its effectiveness and longevity of treatment. When used at rates effective for controlling hydrilla, diquat will also control other native plants actively growing in the treated zone. Because diquat is readily and strongly bound to mineral clays and organic matter, this herbicide is most effective when used in clear water. Use of diquat in turbid water conditions will inactivate the product and result in poor or no control of treated vegetation. The most appropriate use of diquat would be in relatively small-scale, partial lake applications, where broad spectrum control (i.e., removal of most submersed plants) in those settings would only represent a small proportion of the total lake-wide plant community. Guidance for species-selective use will require additional CET studies and linkage with site-specific water exchange conditions on the lake.

### *Endothall*

Good control ( $\geq 80\%$ ) of monoecious hydrilla using endothall should be provided when herbicide exposure times are  $\geq 24$  h, and improved control ( $\geq 90\%$ ) should be achieved at exposure times of  $\geq 48$  h, in the year of treatment. Results of initial CET evaluations indicate that control of shoots from newly sprouted tubers may not be as great as from plants grown from apical shoot cuttings. Therefore, refinement of endothall efficacy against both monoecious and dioecious hydrilla grown from tubers is warranted. When used at rates effective for controlling hydrilla, endothall may also control some native submersed plants if they are actively growing in the treated zone. However, native plants such as pondweeds and coontail can be tolerant of low rates of endothall under cool water temperatures. Herbicide applications should be made in spring when water temperatures are above 12 °C and target plants are actively growing. Caution should be used when applying endothall where vallisneria is a species of concern. Endothall is not affected by water turbidity and can provide plant control in areas protected from high water exchange processes, such as coves and boat marinas. Investigation of higher concentrations with shorter exposure times is needed to determine whether endothall would be effective in areas with



high water exchange on Lake Gaston. Guidance for more effective species-selective use will require additional CET studies and linkage with site-specific water exchange conditions on the lake.

#### *Fluridone*

It is well-established that fluridone is highly effective against dioecious hydrilla at low application rates ( $\leq 20$  ug/L) when aqueous exposure times are extended beyond 45 d. When used at low rates, many native submersed plants are tolerant to fluridone. Still, based upon results of small- and field-scale evaluations, caution should be used when applying fluridone at rates  $\geq 5$   $\mu\text{g ai/L}$  if the native plant, vallisneria, is a species of concern in the treatment area. Results from laboratory CET evaluations have reported that fluridone can provide 90 % control of monoecious hydrilla, and that susceptibility of young plants sprouted from tubers was similar to responses measured in plants grown from apical shoot cuttings. However, these CET trials used high rates of fluridone ( $\geq 100$  ug/L) and short exposure times ( $\leq 4$  d). While fluridone has been used to control monoecious hydrilla on Lake Gaston, documentation of CET relationships with that biotype has never been fully developed. Comparing levels of monoecious hydrilla control with aqueous fluridone residues monitored from operational treatments on the lake would fill a data gap and help determine/verify the CET relationships. This information would be important in providing future guidance for the improved use of fluridone on Lake Gaston.

#### *Carfentrazone-ethyl and Flumioxazin*

These protox inhibitors are a new class of chemistry being evaluated for use in aquatic sites. These products have the potential for offering additional quick-acting herbicides for rapid knockdown of target plants. Under the dose (200 ug/L) and short exposure time (6 h) evaluated, both products provided herbicidal activity against monoecious hydrilla, with carfentrazone showing early growth suppression and flumioxazin showing reduced shoot mass (up to 23 %). However, guidance for using these products on Lake Gaston cannot be provided until further investigations of the interaction between water pH, herbicide dosing rate, and aqueous exposure time are conducted.

*Penoxsulam*

This ALS inhibitor is a new class of chemistry being evaluated for submersed plant control. Initial results indicate that a penoxsulam application rate of 20 ug/L with 90 d of exposure will provide adequate control of monoecious hydrilla. In addition, the minimum effective penoxsulam rate may be lower when a contact herbicide (such as endo-thall) is applied following initial penoxsulam application. This improved activity at lower rates may be explained by a burn-down of shoot foliage by the contact herbicide coupled with regrowth suppression by the systemic compound penoxsulam. Penoxsulam at 10 ug/L or lower -- applied alone -- was generally inadequate for hydrilla control regardless of exposure time. Southern naiad seems to be sensitive to penoxsulam and additional work should be conducted to confirm its response to that non-target plant. Moreover, additional CET evaluations are needed to confirm the findings from this study prior to developing guidance for use of penoxsulam to control monoecious hydrilla on Lake Gaston. These evaluations could include limited field-scale applications in areas of the lake with appropriate water exchange characteristics.

*Imazamox*

This ALS inhibitor is a new class of chemistry being evaluated for submersed plant control. Initial CET trials showed that imazamox provided good control of monoecious hydrilla, particularly at the highest rate tested. Lower imazamox rates generally resulted in less control, and there was some indication that multiple applications of low rates did not provide better control, height reduction, or dry weight reduction than a single application. Imazamox rates of up to 80 µg ai/L show activity and some injury on the native submersed plant, vallisneria, but regrowth of that plant would be expected. Additional CET evaluations are needed to confirm preliminary observations and to evaluate the potential utility of combining low rate imazamox applications with various contact herbicides. Refinement of CET relationships, such as posttreatment regrowth, application rates versus shorter exposure times, and impacts on non-target submersed plants are also required prior to developing guidance for operational use on Lake Gaston.

## **Eurasian Watermilfoil**

### *Diquat*

This product is useful in areas where herbicide contact times are greatly limited (e.g., 3 to 12 h) due to water exchange processes. Extensive treatment experience has shown that one application of diquat at recommended rates can provide greater than 80% knockdown of Eurasian watermilfoil shoots, with regrowth occurring in 6 to 8 wk posttreatment. Since it is a broad-spectrum product, actively growing shoots of non-target submersed plants that occur within the treated zone will also be controlled. Because diquat is readily and strongly bound to mineral clays and organic matter, this herbicide is most effective when used in clear water. Use of diquat in turbid water conditions will inactivate the product and result in poor or no control of treated vegetation. Currently, there are no established CET relationships for diquat to allow for its use as a method for selectively controlling Eurasian watermilfoil. The most appropriate use of diquat for Eurasian watermilfoil control in Lake Gaston would be for relatively small-scale, partial lake applications, where broad spectrum removal of most submersed plants in those settings would only represent a small proportion of the total lake-wide plant community. Guidance for species-selective use will require additional CET studies and linkage with site-specific water exchange conditions on the lake.

### *Endothall*

At recommended treatment rates, exposure time should be maintained for at least 18 to 24 h for best results in controlling Eurasian watermilfoil. Given these exposure times, water in treatment areas should be quiescent, with minimal flow and applications made in areas > 2 ha (5 acres) in size. When used in this manner, there is a rapid kill of plant shoots that results in >80% knockdown within a year of treatment; however, regrowth can occur in 6 to 8 wk. Herbicide applications should be made in spring when water temperatures are above 12 °C and plants are actively growing. Endothall is not affected by water turbidity and can provide plant control in areas protected from high water exchange processes, such as coves, and boat marinas. Endothall is generally considered to be a non-selective herbicide and recommended application rates may impact some native submersed vegetation. Caution should be used when applying endothall where vallisneria is a species of concern. However, small-scale studies have shown that low rates of endothall applied in early spring with exposure times of 1 to 3 d,

can be efficacious against Eurasian watermifoil with minimal damage to non-target vegetation. Investigation of higher concentrations with shorter exposure times is needed to determine whether endothall would be effective in areas with high water exchange on Lake Gaston. Guidance for more effective species-selective use will require additional CET studies and linkage with site-specific water exchange conditions on the lake.

#### *2,4-D and Triclopyr*

These auxin-like products are very effective and selective for controlling Eurasian watermilfoil ( $\geq 85\%$  control). They are most effective when aqueous herbicide contact times can be maintained for  $\geq 18$  h. Partial lake treatments using 2,4-D or triclopyr would include moderately-sized application blocks of 2 to 4 ha (5 to 10 acres) or all areas in the littoral zone infested with Eurasian watermilfoil, where water exchange processes fit the known CET relationships for the products. These herbicides are most efficacious when applied to young, actively growing plants. When used in the appropriate settings, Eurasian watermilfoil may be controlled for up to three years, including the year of treatment, with no adverse impacts to native vegetation. Nonetheless, plant regrowth may occur in 4 to 6 wk if Eurasian watermilfoil is not completely killed during the initial herbicide applications. Since 2,4-D and triclopyr are selective for dicots, exposure to even the highest allowable rate of these herbicides should cause little or no injury to a monocot such as vallisneria. While CET relationships are well understood for 2,4-D and triclopyr, linking water residues with target plant control during operational use of these products on Lake Gaston will verify the CET relationships and provide future guidance for the improved use of these auxin-like materials on the lake.

#### *Fluridone*

This systemic product provides excellent control of Eurasian watermilfoil at very low rates ( $\leq 10$  ug/L), when appropriate contact times are met (45 to 60 d exposure). Susceptible plants die and decompose slowly, with  $>90\%$  knockdown within a year of treatment, and target plant regrowth usually does not occur for over 12 months. Low rates are selective for Eurasian watermilfoil with minimal injury to non-target plant species. Fluridone efficacy is best provided with whole lake treatments, or very large treatment blocks,  $\geq 40$  ha (100 acres), where water exchange processes are limited. While CET relationships are well understood for fluridone, linking water residues with target plant control during operational use of this product on

Lake Gaston will verify fluridone/Eurasian watermilfoil CET relationships and provide future guidance for the improved use of this herbicide on the lake.

#### *Carfentrazone-ethyl and Flumioxazin*

These protox inhibitors are a new class of chemistry being evaluated for use in aquatic sites. These products have the potential to become additional quick-acting herbicides for rapid knockdown of target plants. Because these products are contact herbicides, Eurasian watermilfoil regrowth would be expected after one season of control. Carfentrazone-ethyl is considered to be a selective herbicide in terrestrial agriculture, and has the potential for selective control of Eurasian watermilfoil in aquatic systems. While carfentrazone-ethyl shows good activity and control on Eurasian watermilfoil, more refinement of CET relationships and limited studies are required for a better understanding of the product's performance in the field. Flumioxazin applied at a high rate showed good control ( $\geq 90\%$ ) of Eurasian watermilfoil. However, additional CET evaluations in combination with a component to determine shoot regrowth potential following treatment should be conducted. This information will be necessary prior to developing guidance for using these protox inhibitor compounds to control Eurasian watermilfoil in Lake Gaston.

#### *Bispyribac-sodium*

This ALS inhibitor is a new class of chemistry being evaluated for submersed plant control. Initial results indicate that bispyribac-sodium is efficacious against Eurasian watermilfoil and application rates of  $\geq 20 \mu\text{g ai/L}$  for extended exposure periods (11 wk) can provide  $\geq 90\%$  control of that target plant. Bispyribac-sodium rates of up to  $80 \mu\text{g ai/L}$  show activity and some injury on the native submersed plant, vallisneria, but regrowth of that plant would be expected. Refinement of CET relationships, such as posttreatment regrowth, application rates versus shorter exposure times, and impacts on non-target submersed plants are required prior to developing guidance for operational use on Lake Gaston.

#### *Imazamox*

This ALS inhibitor is a new class of chemistry being evaluated for submersed plant control. Initial results indicate differential response and activity of imazamox against Eurasian watermilfoil, and that high rates

(100 to 200 ug/L) can provide up to 72 % control of that target plant. Additional studies might focus on how imazamox could be used as a plant growth regulator in some situations. Imazamox rates of up to 80 µg ai/L show activity and some injury on the native submersed plant, vallisneria, but regrowth of that plant would be expected. However, refinement of CET relationships, such as posttreatment regrowth, application rates versus shorter exposure times and impacts on non-target submersed plants are required prior to developing guidance for operational use on Lake Gaston.

#### *Penoxsulam*

This ALS inhibitor is a new class of chemistry being evaluated for submersed plant control. Initial results indicate that penoxsulam applied at rates > 10 µg ae L<sup>-1</sup> and for exposure periods of 45 d can provide up to 87 % control of Eurasian watermilfoil. Southern naiad seems to be sensitive to penoxsulam and additional research should be conducted to confirm its response to that non-target plant. Refinement of CET relationships, such as posttreatment regrowth, application rates versus shorter exposure times, and impacts on non-target submersed plants are required prior to developing guidance for operational use on Lake Gaston.

#### **Egeria**

Egeria has proven to be a very difficult plant to control with herbicides. The contact herbicide, diquat and/or diquat combined with a chelated copper, have been the products of choice for most egeria control programs. The most appropriate use of diquat for egeria control in Lake Gaston would be for relatively small-scale, partial lake applications, when plants are young and actively growing in non-turbid water conditions where broad spectrum removal of submersed aquatic plants in those settings would only represent a small proportion of the total lake plant community. Other products that may have the potential to control egeria include the contact herbicides, carfentrazone-ethyl and flumioxazin, and the systemic products, fluridone, penoxsulam, imazamox, and bispyribac-sodium. However, the establishment of CET relationships against egeria is required prior to developing guidance for operational use of these products on Lake Gaston.

#### **Lyngbya**

The bluegreen alga, lyngbya, is also a very difficult organism to control using chemicals, and there is a growing need for industry to develop and

evaluate algaecides to control this invasive species. Copper products have been the chemicals of choice in lyngbya control programs, but efficacy has been inconsistent. Results from initial studies have not indicated increased efficacy against lyngbya when combining chelated copper products with endothall. And while results from these studies have shown that lyngbya is susceptible to some forms of chelated copper, repeat applications may be required to provide adequate control of this nuisance alga. The identification of CET relationships in yet-to-be-identified new products is required prior to developing guidance for the use of lyngbya control chemicals on Lake Gaston.

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14. ABSTRACT <p>Lake Gaston is a large, multiple purpose impoundment on the Roanoke River between eastern North Carolina and Virginia. Invasive plants have been increasing on the lake since 1982. By 2002, over 1,200 ha of the lake were infested with several invasive plants, and an integrated management program (herbicides and grass carp) was underway. To improve herbicide performance on the lake, this study focused on three phases for controlling the lake's invasive submersed vegetation (the plants targeted were monoecious and dioecious hydrilla; Eurasian watermilfoil; egeria; and the bluegreen alga, lyngbya; non-target plants evaluated were vallisneria and southern naiad.) Phase one summarizes herbicide dose-response interactions (concentration and exposure time (CET) relationships) for controlling these plants using older aquatic herbicides; phase two evaluates CET relationships for new aquatic herbicides; and phase three provides interim management guidance for Lake Gaston.</p> <p>Product-specific CET information is best utilized when combined with site-specific water exchange patterns found in plant stands targeted for chemical applications. Prescriptive treatments can then be developed to selectively remove invasive plants. Results from evaluations showed that control of target plants was dependent upon product specific herbicide CET relationships, with efficacy ranging from poor to excellent.</p> <p style="text-align: right;">(continued)</p>					
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**14. ABSTRACT**

Information provided in this report can be used for developing prescriptive treatment strategies for selectively controlling invasive plants on Lake Gaston. Recommendations for specific herbicides should be viewed as a “best fit” based on current information. This interim chemical control guidance should be refined once site-specific water exchange processes are determined for treatment sites on Lake Gaston.